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RADIAL INCLUSIONS OF GIANT CELLS

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Lesions simulating tuberculous or foreign body granulation tissues and containing giant cells with rosette-shaped inclusions have been observed in the human liver, lungs, spleen and lymph nodes; in subcutaneous tissues about paraffin; in scar tissues around the ducts of the mammary gland dilated with retained secretion; in the wall of dermoid cysts; in adenomyoma nodules of the uterus; in nodules of the myocardium; in scars of the capsule of the spleen; in chronic tuberculous lesions, and in certain chronic inflammations of the skin and subcutaneous tissues. The nature of these radial inclusions in the giant cells has been an enigma, and many divergent opinions have been recorded regarding their composition and the significance of the associated lesions. The historical details are reviewed later, and the immediately succeeding paragraphs describe these granulation tissue lesions in the lungs, spleen or parabronchial lymph nodes of ten bodies and in twenty-six specimens of tissues removed surgically and submitted to routine microscopic examination. The lesions with the giant cells and their radial inclusions in the specimens of tissues removed surgically were incidental and presumably had no relation to the disorders that impelled the operations.

AUTOPSY MATERIAL

AUTOPSY 1.—A white man, aged 72, with a chronic infection and concretions of the urinary tract, had taken olive oil and other simple remedies by mouth. The spleen, weighing 290 Gm., had many fibrous nodules 3 to 20 mm. in diameter. These were fibrous granulation tissues containing confluent and discrete structures resembling tubercles. There were many foreign body giant cells, epithelioid cells, lymphocytes and plasma cells. The giant cells had vacuoles from 6 to 7 microns in diameter with minute spherical granules. Many of the giant cells contained one or two radial inclusions from 5 to 20 microns in diameter (fig. 1). A radial structure 15 microns in diameter appeared to be extracellular in one section.

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Fibrous tissues around some of the bronchi and blood vessels of the lungs also contained giant cells with radial inclusions, and aggregates of pulmonary alveoli were filled with vacuolated fibrin and mononuclear exudate cells (fig. 2). Droplets of oil were demonstrated in such tissues with sudan III. The tissues in parabronchial lymph nodes were similar to those in the fibrous nodules of the spleen.

AUTOPSY 2.—A white woman, aged 50, died fifteen hours following thyroidectomy. In the fibrous tissues along a few of the blood vessels and bronchioles and in the parabronchial lymph nodes were giant cells with radial inclusions associated with a few lymphocytes and mononuclear exudate cells. There were no lesions in the liver and spleen.

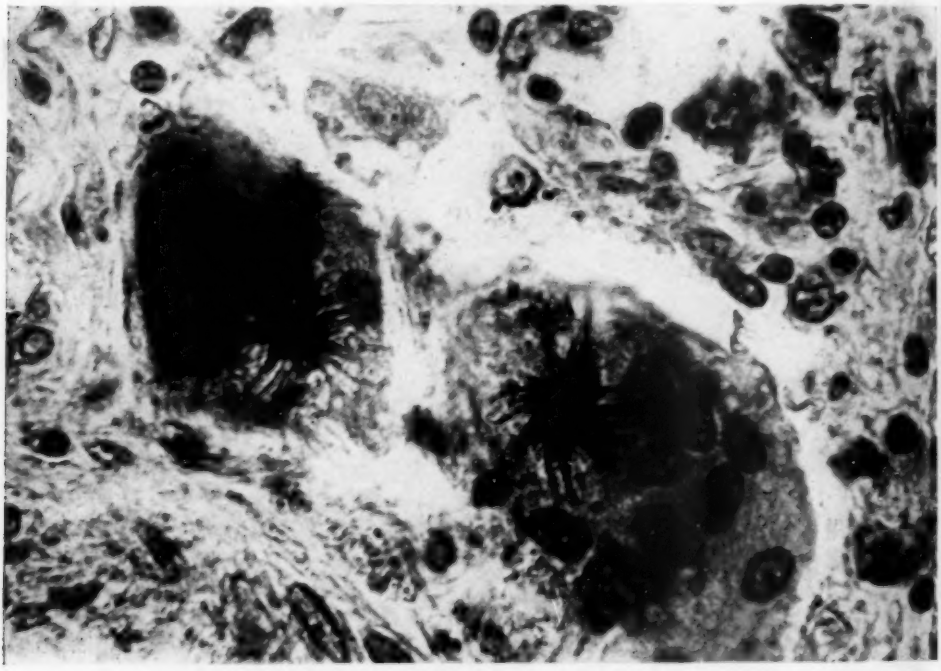

Fig. 1.—Photomicrograph illustrating giant cells with radial inclusions (autopsy 1). Magnification, × 1,260.

AUTOPSY 3.—A white man, aged 70, treated about five years for pernicious anemia, died of uremia. Small clusters of foreign body giant cells with radial inclusions were noted about the blood vessels of the lungs. The liver, spleen and lymph nodes had none.

AUTOPSY 4.—A white man, aged 47, died of carcinoma of the pancreas. One of the lungs had subpleural scars containing chronic cellular exudates, and a few multinucleated foreign body giant cells in granulation tissues with cholesterol clefts. Some of the giant cells had radial inclusions. The parabronchial lymph nodes, spleen and liver had no giant cells.

AUTOPSY 5.—Routine microscopic examination of the tissues in the body of a white man, aged 46, who had had hypertension and clinically and anatomically a spontaneous cerebral hemorrhage on the right, disclosed a few large vacuolated

giant cells with stellate inclusions in the parabronchial lymph nodes, but none in the sections of the lungs and other tissues examined.

AUTOPSY 6.—A white man, aged 72, who had had diabetes for many years, died of acute generalized peritonitis following perforated chronic ulcerative cholecystitis. He had used liquid petrolatum for constipation and an oil spray for catarrh of the nose. Routine microscopic examination of the lungs disclosed vacuolated exudates in the alveoli and bronchi. Along the margin of the vacuoles were foreign body giant cells. Similar clusters of vacuoles were discovered in the periportal tissues of the liver, in the sinusoids of the spleen and in the parabronchial lymph nodes. Sections of the spleen and liver suitably stained demonstrated that these vacuoles originally contained an oil. The tissue reaction about these droplets of oil was

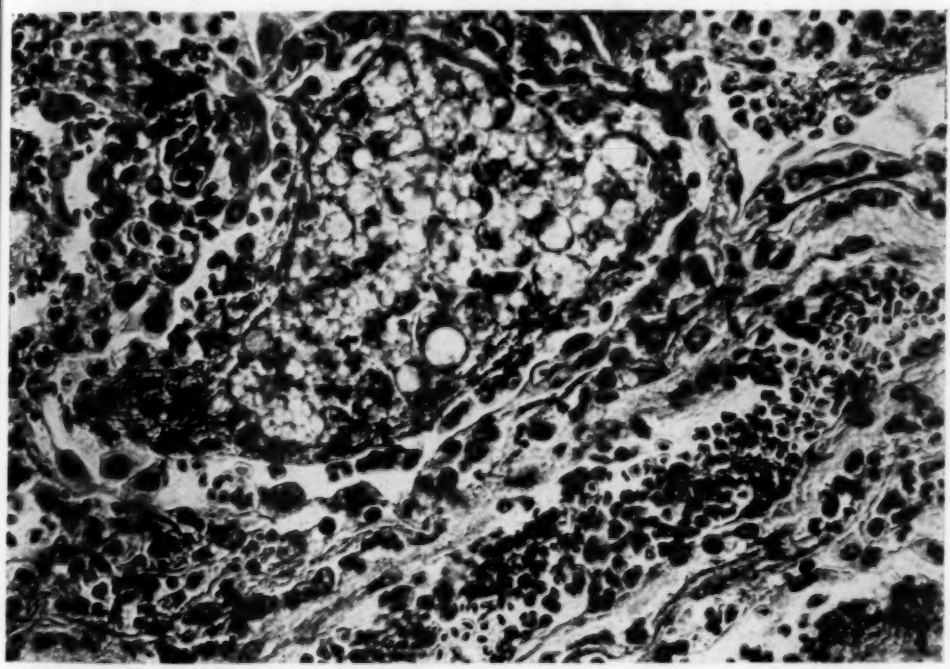

Fig. 2.—Photomicrograph illustrating the vacuolated exudates in the lungs in autopsy 1, presumably where droplets of liquid fat had been dissolved during the staining procedures. Magnification, × 620.

slight in the various places mentioned, but in the parabronchial lymph nodes were a few foreign body giant cells with large vacuoles and a large stellate inclusion.

AUTOPSY 7.—An emaciated white woman, aged 39, for many years had had pulmonary tuberculosis, mainly of the left lung. Spatially distributed over three months and seven days to suit the physical conditions of the patient, three thoracoplastic operations were performed on the left posterior region and two on the anterior region. Within a few hours after the last operation the patient became unconscious, dyspneic and cyanotic and slowly died. Droplets of fat were demonstrated in the capillary and precapillary bed of the right pulmonary artery and in dilated sinusoids of the liver at the periphery of some of the lobules. The parabronchial lymph nodes had focal masses of epithelioid cells resembling tuber-

culous nodules or tissue reactions caused by fat. Apart from these lesions were a few vacuolated foreign body giant cells with stellate inclusions.

AUTOPSY 8.—A Negro, aged 67, died of an infarct (thrombosis) of the brain. There was hyperplasia of the large mononuclear cells in the parabronchial and biliary (?) lymph nodes. These lymph nodes contained also a few widely scattered giant cells, some with stellate inclusions.

AUTOPSY 9.—A Negro boy, aged 8 years, died of acute confluent bronchopneumonia. In the upper pole of the spleen was an encapsulated mass of necrotic tissue 4 by 0.8 by 0.8 cm. In the necrotic tissue débris were many mossy crystals with golden-brown pigment and of the size mentioned. There was no granulation tissue reaction about them.

AUTOPSY 10.—The tissues of a biliary lymph node (supplied by Dr. E. R. Le Count) contained clusters of large and small vacuoles. About the vacuoles were foreign body giant cells, some with a stellate inclusion. The fibroblastic tissue reaction was slight.

SURGICAL MATERIAL

SPECIMEN 1 (*Lymph Node*).—A housewife, aged 58, had noted a painless lymph node in the left supraclavicular fossa of the neck increase within a year to 4 by 3 by 2 cm. The skin was not involved, and three other nodes, freely movable, were 1 cm. in diameter. The large node was removed. The tissues were tuberculous, but also contained vacuolated giant cells, a few with one or two radial inclusions. The scar tissues had invaded the fat around the lymph node.

SPECIMEN 2 (*Thyroid Tissues*).—Thyroid tissues weighing 90 Gm. with marked glandular hyperplasia were removed from a man, aged 45. In one block was a mass of scar and granulation tissues containing cholesterol slits, and a few vacuolated giant cells with radial inclusions. One radial crystal seemed to be extracellular.

SPECIMEN 3 (*Thyroid Tissues*).—Thyroid tissues with marked glandular hyperplasia and weighing 102 Gm. were removed from a white woman, aged 47. In chronic granulation tissues from a necrotic portion were mononuclear phagocytes with blood pigment and several giant cells with radial inclusions.

SPECIMEN 4 (*Thyroid Tissues*).—Thyroid tissues weighing 94 Gm., a recurrent goiter, were removed from a Negress, aged 54. There was glandular hyperplasia with recent and old hemorrhages. The old hemorrhages had organized into scars containing mononuclear phagocytes with blood pigment, granular débris with cholesterol slits and a few foreign body giant cells, some with radial inclusions.

SPECIMEN 5 (*Thyroid Tissues*).—A fluctuant mass of thyroid tissue weighing 60 Gm. was removed from a Negress, aged 41. It was mainly an adenoma altered by hemorrhages, edema, necrosis and organization. The granulation tissues contained multinucleated foreign body giant cells, some with radial inclusions; also, coarse green and yellow threadlike masses (iron-encrusted fibers or iron-salt precipitates [1]).

SPECIMEN 6 (*Thyroid Tissues*).—Thyroid tissues from a white woman, aged 58, weighed 86 Gm. Granulation tissues in an adenoma contained tissue débris with cholesterol slits and giant cells with stellate inclusions.

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1. Askanazy, M., and Bamatter, F.: Centralbl. f. allg. Path. u. path. Anat. **43**:337, 1928.

SPECIMEN 7 (*Thyroid Tissues*).—Thyroid tissues weighing 255 Gm. were removed from a white woman, aged 45. There were diffuse and focal regions of glandular hyperplasia. One of the latter, 6 cm. in diameter, was necrotic, hemorrhagic and partially replaced by granulation tissues. In the granulation tissues were bizarre fibrillar structures encrusted with lime or blood pigment, and among these were foreign body giant cells, some with one or more stellate inclusions.

SPECIMEN 8 (*Mammary Gland*).—A woman, aged 50, had noted a lump in the breast for five years. A retention cyst 2.5 cm. in diameter, and several others, 3 or 4 mm. in diameter, were in the excised tissues. A brown mass 3 mm. in diameter in one of the small cysts contained granulation tissues with cholesterol slits and foreign body giant cells with radial inclusions from 15 to 20 microns in diameter.

SPECIMEN 9 (*Tonsil*).—In a crypt of a tonsil from a white man, aged 30, was a 2 mm. mass of chronic granulation tissue with vacuolated giant cells containing radial inclusions.

SPECIMEN 10 (*Fractured Bone*).—A gardener, aged 40, fractured the os lunatum of his left wrist. After four months the bone was removed in several pieces. In some dense scar tissues were a few foreign body giant cells with stellate inclusions.

SPECIMEN 11 (*Uterus*).—The body of the uterus and the left fallopian tube with hydrosalpinx were removed from a multiparous white woman, aged 33. The endometrium was from 2 to 4 mm. thick and had recent hemorrhages. In the interstices between the smooth muscle bundles of the stratum submucosum were small masses of lymphoid tissue containing foreign body giant cells with radial inclusions. These lesions extended from 1 to 2 mm. beneath the endometrium.

SPECIMEN 12 (*Sebaceous Cyst*).—Three sebaceous cysts, from 8 to 20 mm. in diameter, were removed from the scalp of a man, aged 48. Along the margin of one was a mass of foreign body granulation tissue containing cholesterol slits, some giant cells with granular inclusion material and a few vacuolated giant cells with a large radial inclusion.

SPECIMEN 13 (*Dermoid Cyst of the Ovary*).—A dermoid cyst of the ovary was removed from a Negress, aged 56. A mass of chronic granulation tissue in the wall contained mononuclear phagocytes with brown blood pigment, many large vacuolated cells and several vacuolated giant cells with stellate inclusions.

SPECIMEN 14 (*Dermoid Cyst of the Ovary*).—In the wall of a dermoid cyst of the ovary, along the edges of large vacuoles such as remain where an oil has been dissolved, were vacuolated foreign body giant cells with one or more radial inclusions.

SPECIMEN 15 (*Scar Tissues*).—Scar tissues of a urinary cystotomy fistula contained foreign body giant cells with refractile suture material and others vacuolated with one or two radial inclusions, the largest 35 microns in diameter.

SPECIMEN 16 (*Scar Tissues*).—Scar tissues from another urinary cystotomy fistula had similar giant cells. One radial inclusion was 20 microns in diameter.

SPECIMEN 17 (*Scar Tissues*).—Painful "neuroma" scars in the stump of a left forearm amputated twenty-five years before contained nerve fibers and focal exudates, including one or more foreign body giant cells, some vacuolated and with radial inclusions from 3 to 20 microns in diameter.

SPECIMEN 18 (*Scar Tissues*).—The uterus from a woman aged 43 was rough with adhesions following a preceding laparotomy. Peritoneal scars from 1 to 5 mm. in diameter contained epithelioid cells, a few plasma cells and leukocytes,

some giant cells with suture material and others with stellate inclusions 15 microns in diameter.

SPECIMEN 19 (*Scar Tissues*).—An old laparotomy scar adherent to the omentum was excised from a woman, aged 30. The scarred fat tissues contained foreign body giant cells with suture material inclusions and a few with radial inclusions.

SPECIMEN 20 (*Scar Tissues*).—Scar tissues removed from an old empyema of the chest of a man, aged 39, contained large vacuoles. Along the margin of the vacuoles were multinucleated giant cells, some vacuolated, others with stellate inclusions.

SPECIMEN 21 (*Scar Tissues*).—A fistula of the right epididymis in a man, aged 40, had drained for sixteen years. The excised granulation and scar tissues contained a few giant cells with refractile suture material and a few vacuolated with a single radial inclusion.

SPECIMEN 22 (*Scar Tissues*).—Nodules from the peritoneum of a Negress, aged 38, contained fat and metastatic glandular carcinoma. A mass of foreign body granulation tissue encompassed granular débris with many cholesterol clefts. There were numerous mononuclear phagocytes with brown blood pigment and clusters of foreign body giant cells. A number of these contained small stellate inclusions.

SPECIMEN 23 (*Scar Tissues*).—Periarticular fat removed from a woman about seven years after an injury to her knee contained regions of necrotic fat and granular débris. The granulation tissues around the necrotic tissues had many mononuclear phagocytes, some vacuolated and others with granular blood pigment and mossy golden-brown rosettes 10 microns in diameter. Other rosettes, from 20 to 30 microns in diameter, were encompassed by large epithelioid cells.

SPECIMEN 24 (*Scar Tissues*).—Old scar and fat tissues about fistulas of osteomyelitis of the right femur contained a few foreign body giant cells with radial inclusions.

SPECIMEN 25 (*Paraffin Scar Tissues*).—Scars of the face caused by injections of paraffin (?) seven years before excision contained large and small vacuoles. Along the margins of the vacuoles were foreign body giant cells, a few with stellate inclusions.

SPECIMEN 26 (*Paraffin Scar Tissues*).—Paraffin injected into the right groin of a man, aged 43, failed to correct an inguinal hernia. The scar tissues about these masses of paraffin contained large and small vacuoles, and along the edges of the vacuoles were foreign body giant cells, some with radial inclusions.

HISTORICAL RÉSUMÉ

Goldmann,[2] in 1890, seems to have written the first description of radial inclusions in giant cells. He saw them in histologic preparations of the wall of a dermoid cyst of the neck taken from a man, 25 years old. Ribbert examined the wall of a cyst of an omentum that Ris [3] had removed and noted in the tissues clefts such as remain where cholesterol has been dissolved and giant cells with radial inclusions. The same year, De Buck and Broeckaert [4] described inflamma-

2. Goldmann, E. E.: Beitr. z. path. Anat. u. z. allg. Path. **7**:553, 1890.

3. Ris, F.: Beitr. z. klin. Chir. **10**:423, 1893.

4. De Buck, D., and Broeckaert, J.: Bull. Acad. roy. de méd. de Belgique **17**:890, 1903.

tory reactions around paraffin in the tissues of a youth, 17 years of age. They illustrated radial inclusions within giant cells like those described by Goldmann and Ris.

Wolbach,[5] in 1911, published abstracts on five postmortem examinations in which visceral masses of chronic granulation tissue containing giant cells with radial inclusions had been observed. The first postmortem examination was that of the body of a woman, aged 65, who died fifteen days after resection of a carcinoma of the colon. In masses of fibrous tissue along the interlobular septums of the lungs and about the bronchi and large vessels were giant cells with radial inclusions. The second examination was that of the body of a man, aged 72, with chronic urinary cystitis and carcinoma of the neck which was considered metastatic from a primary growth in the nasopharynx. Giant cells with radial inclusions were present only in the parabronchial lymph nodes. In the lungs of another man, 39 years old, miliary lesions contained giant cells with radial inclusions. Similar giant cells were in the bronchial lymph nodes. The spleen, the liver and the lymph nodes of the neck, mesentery and prevertebral tissues of a woman, aged 49, with exophthalmic goiter, had lesions containing giant cells with stellate inclusions. In the fifth instance of visceral lesions in Wolbach's report the lesions were in the lungs, spleen and liver of a woman, aged 49. Death was caused by a cerebral hemorrhage. In his discussion Wolbach referred to similar cell inclusions in tissues of a giant cell sarcoma furnished by Dr. F. B. Mallory. Many giant cells in this preparation contained one or several hyaline spherules with radiating, delicate curved and straight spines.

Vogel,[6] in 1911, reported lesions containing giant cells with radial inclusions in the lungs of a boy, aged 11 years. Scattered in both lungs, almost completelv airless, were small gray tough nodules and bands resembling miliary tubercles. Their distribution corresponded to the terminal branchings of the smallest bronchioles. No tuberculous lesions were present. Histologic examination demonstrated desquamated alveolar epithelium and obliterated terminal bronchioles. Only segments of the larger bronchioles remained; other portions were replaced by connective tissue which extended into the lumens. Giant cells with one or more radial inclusions were in the perivascular and peribronchial connective tissues. Vogel reported these as unusual foreign body giant cells with bronchiolitis obliterans. Cyanosis, dyspnea and a dry cough were some of the clinical symptoms.

In discussing the obliteration of ducts in the mammary gland and the retention thereby of fatty substances, Letulle[7] mentioned the presence of giant cells with stellate inclusions in granulation tissues around the gland tubules. Iwanzoff[8] observed lesions containing giant cells with radial inclusions in a large adenomyoma of the uterus of a woman aged 50. Some of the larger myoma nodules contained focal lesions the size of a submiliary tubercle, composed of lymphocytes and giant cells with radial inclusions. Ernst[9] noted giant cells with radial inclusions in a preparation mounted in balsam, which he said resembled those described by Wolbach and others. Hummel[10] observed many nodules of chronic granulation tissue with giant cells containing radial inclusions in the lungs and a few in the spleen of a woman, aged 52, who had worked for many years in a pottery

5. Wolbach, S. B.: J. M. Research **24**:243, 1911.
6. Vogel, Karl: Virchows Arch. f. path. Anat. **206**:157, 1911.
7. Letulle, M.: Rev. de gynéc. et de chir. abd. **19**:401, 1912.
8. Iwanzoff, P.: Beitr. z. path. Anat. u. z. allg. Path. **52**:202, 1912.
9. Ernst, Paul: Beitr. z. path. Anat. u. z. allg. Path. **53**:429, 1912.
10. Hummel, Edward: Virchows Arch. f. path. Anat. **211**:173, 1913.

shop. A chronic cough present before she engaged in this occupation became worse. Firket[11] saw giant cells with asteroid inclusions and cholesterol clefts in the connective tissues of an encapsulated colloid carcinoma of the neck and also in the tissues of the nose around masses of paraffin. Kranzfeld[12] reported fibrous nodules containing scar tissue and many multinucleated giant cells with radial inclusions in the capsule of the spleen of a man, aged 20, who died with acute generalized peritonitis secondary to appendicitis. Herxheimer and Roth,[13] in their analysis of the finer structure and genesis of epithelioid cells and giant cells in tuberculosis, commented briefly on giant cells with radial inclusions. They had observed these cells once in chronic tuberculous tissues, twice in dermoid cysts of the ovary in parts of the wall containing large cells and giant cells filled with fat and lipoids, which suggested a pseudoxanthoma, and once in lupus pernio of the skin.

Diss[14] recorded two lesions containing giant cells with radial inclusions. One was a subcutaneous node that developed slowly and without ulceration of the skin in the forehead of a woman, aged 65. The excised tissues consisted of inflammatory nodules which had elevated and thinned the skin and had infiltrated the entire thickness of the frontal muscle. In the second instance there were nodules in the myocardium of a woman, aged 46, who had died of pulmonary tuberculosis. Diss, in his report, referred to an observation by P. Masson of similar giant cells with radial inclusions. An unsuccessful search for a record of this observation in the usual indexes of the medical literature prompted a letter of inquiry to Dr. Masson. In his reply he stated: "When my assistant, Dr. Diss, was preparing his report, I showed him various tissues containing giant cells with radial inclusions in my own personal collection, and in particular a cancer of the breast with an abundance of these cells in an old hemorrhage in the process of resorption. This is the cancer to which Diss has alluded, but I have never published an account of the observation. I have seen these asteroid bodies several times and always within a necrotic hemorrhagic focus, and thus in the presence of disintegrating blood which to me seems indispensable for their production."

Frothingham,[15] in 1929, in a report of progressive thrombosis of the smaller branches of the pulmonary arteries in a woman, aged 38, described tubercle-like lesions of mononuclear and giant cells in the pleura and interlobular septums adjacent to blood vessels and bronchi. Some giant cells contained the stellate inclusions described by Wolbach. The histology and distribution of the lesions in the lungs were identical with those in Wolbach's report. Similar lesions were in the liver. Two illustrations of giant cells with radial inclusions appear in Mallory's text.[16] He stated that "spiculated bodies" had been seen in the spleen in a few instances, enclosed in endothelial leukocytes and giant cells. The lesions resembled miliary tuberculosis but were without necrosis. The bodies seemed chemically fibrinoid. They were not the cause of the lesion, which was probably of infectious origin, but were a secondary formation. Stoddard and Cutler[17] observed in

11. Firket, C.: Virchows Arch. f. path. Anat. **215**:454, 1914.

12. Kranzfeld, M.: Frankfurt. Ztschr. f. Path. **15**:297, 1914.

13. Herxheimer, G., and Roth, W.: Beitr. z. path. Anat. u. z. allg. Path. **61**:1, 1916.

14. Diss, A.: Bull. et mém. Soc. anat. de Paris **94**:349, 1924.

15. Frothingham, Channing: Am. J. Path. **5**:11, 1929.

16. Mallory, Frank B.: Principles of Pathological Histology, Philadelphia, W. B. Saunders Company, 1914, pp. 207 and 613.

Rockefeller Institute for Medical Research, 1916, p. 55.

17. Stoddard, James, and Cutler, E. C.: Torula Infection in Man, Monogr. 6,

Torula-infected lung tissues giant cells with stellate masses, which they considered the same as those described by Wolbach in the spleen.

The accounts mentioned state that the lesions containing giant cells with radial inclusions are essentially chronic granulation tissues like those caused by a foreign body. The dimensions of the giant cells vary, but are about the same as those of the Langhans' giant cells in tuberculous lesions. The cytoplasm of the giant cells is vacuolated, and radial inclusions are not present in all. The inclusions seen by Ris were from 15 to 25 microns in diameter; those seen by Wolbach, from 5 to 25 microns in diameter. Fine or coarse spines radiate from a central, compact, round, oval or elongated nucleus; these are from fifteen to thirty in number, according to Vogel, or less (Wolbach). When the number is small and the inclusion large, the spines are coarse; when there are many, they are fine. A single giant cell may contain one or several. The inclusions generally are found in a clear part of the cytoplasm. The spines of some radial inclusions extend straight from the center; others are curved distally with ends bent, as though the inclusions were confined in a place less than their diameter. Wolbach alone reported extracellular radial structures.

The nature or origin of the radial inclusions is not definitely stated in various reports. Most authors limit themselves to descriptions of the appearance of the structures, the staining qualities and solubility reactions. The conclusion that all the reports concern radial structures of the same type is presumptive, but the illustrations and descriptions favor the opinion that, although they are present in various parts of the body, they are essentially the same. The original description by Goldmann referred to the inclusions as fat crystals, an opinion rather than a conclusion derived from tests. Ribbert, in the report by Ris, is quoted as having stated that the inclusions were not crystals or echinococcus hooklets but resembled molds (undeveloped spores and filaments). De Buck and Broeckaert considered them hypertrophied centrosomes or asters.

Wolbach tested the solubility of the radial inclusions in acids and alkalis and found them insoluble. He noted that the radial inclusions in formaldehyde-fixed material were not blackened with silver nitrate, were not colored with iron-hematoxylin and Weigert's elastic tissue stain and did not react with fat stains. No qualitative test for iron was obtained. The best stain was Mallory's phosphotungstic acid-hematoxylin method, which colored them dark purple. Because of this staining quality, Wolbach discussed their formation from fibrin or fibrin derivatives, but did not state definitely that this was their origin. Although Wolbach observed some of the radial structures in the sinuses of lymph nodes, he concluded because of the rarity of this and because

the small forms were in mononuclear cells and the larger only in giant cells that there was an actual increase in the size of the inclusions after they were taken up by endothelial cells, and that giant cells formed as the inclusions increased in size. Vogel used many dyes and tests in trying to determine the nature of the inclusions. He observed that only elastin stains were effective. They failed to react with stains for fat, amyloid, iron and glycogen. They were insoluble in acids and alkalis and were not doubly refractive. The Bielschowsky stain was mentioned, but the results with this technic were not recorded.

Vogel, in speculating on the cause of the bronchiolitis in his patient, wondered whether some irritant had been aspirated, causing the scarring and pneumonia, and then had crystallized in the lungs, stimulating the formation of giant cells. The medication given at home was linden tea and cod liver oil. This search for clues being unsatisfactory, Vogel considered that possibly the inclusions were derivatives of elastin, but concluded finally that they consisted of a substance with staining reactions like elastin. He also was undecided whether the radial inclusions as foreign bodies stimulated the giant cells or were crystalline products within the cells. The report by Ernst is limited to the examination of a tissue preparation mounted in balsam. He thought that the radial inclusions might be cholesterol. Letulle considered the inclusions parts of engulfed elastic tissue fibers. Iwanzoff, following a discussion of various possibilities, believed some degenerative product of cells the most likely origin of the inclusions. Then, after a comment on the similarity between Wolbach's illustrations and those by Wakabayashi [18] of the astrospheres in tuberculous and other giant cells, he expressed the conviction that the inclusions were degenerated astrospheres. His material did not permit him to confirm this idea. Aschoff,[19] commenting briefly on Iwanzoff's report, disagreed with Ribbert's opinion that the inclusions were undeveloped portions of a mold, but offered no opinion of his own. Hummel, from the results of staining reactions, concluded that the radial inclusions were crystalline precipitates that resembled changed elastin. Certain differences in staining reactions led him to believe that they were not elastin fibers. The "foamy" structure of the giant cells further suggested to Hummel that the origin of the radial inclusions was probably the crystallization of some unidentified substance in the cell. Firket concluded that the radial inclusions were not a retrogressive product of elastic fibers but had arisen in the giant cells through a differentiation of the mitomes, which differentiation was related to the vacuolation of the protoplasm. Kranzfeld believed that these radial structures were probably the result of a disturbance in karyokinesis or

18. Wakabayashi, T.: Virchows Arch. f. path. Anat. **204**:421, 1911.

19. Aschoff, L.: Beitr. z. path. Anat. u. z. allg. Path. **52**:444, 1912.

represented pathologic astrosphere formations. Herxheimer and Roth stated that in a degenerative process of the giant cells the protoplasm becomes fluid and vacuolated. Substances probably protein in nature with the staining qualities of elastin crystallize as radial structures about a nucleus which is some other substance—possibly lipin material, liberated by the changes in the protoplasm. The idea of a lipin substance acting as a nucleus was derived from the observation that some of the radial structures had a central compact granule which could be stained by the Fischler and the Lorraine-Smith methods. Diss expressed the belief that the asteroid bodies resulted from the precipitation of a protoplasmic substance consequent to changes in the colloidal equilibrium of cellular protoplasm.

An analysis of the places in the body with lesions containing giant cells with radial inclusions discloses that they are: (1) focal in tissues abundant with fat (Goldmann, Ris, De Buck and Broeckaert, Ernst[?], Letulle, Firket, Diss, Kranzfeld [?], Herxheimer and Roth); and (2) systemic in visceral tissues such as the lungs, spleen, liver and lymph nodes (Wolbach, Vogel, Hummel, Frothingham). The anomalous places where they have been observed are an adenomyoma of the uterus (Iwanzoff) and the myocardium (Diss). The distribution of the lesions in the lungs is in the peribronchial or bronchial tissues (Wolbach, Vogel, Frothingham); in the spleen, within the pulp tissues (Wolbach), and in the liver, along the portal canals (Wolbach, Frothingham).

In summary, the inclusions are reported to be insoluble in potassium hydroxide and mineral acids (Wolbach, Iwanzoff, Vogel, Herxheimer and Roth) and in all the reagents used in fixing, embedding and staining the tissues. They do not react with scarlet red (Wolbach, Herxheimer and Roth); osmic acid (Wolbach); nile blue and sudan III (Hummel, Herxheimer and Roth); the Fischler and Lorraine-Smith methods, excepting the central body (Herxheimer and Roth), and fat stains not specifically mentioned (Vogel). They do not contain iron (Wolbach, Vogel), amyloid and glycogen (Vogel) or mucin (Hummel). They are isotropic in polarized light (Vogel, Herxheimer and Roth); they do not reduce (formaldehyde-fixed tissue) silver nitrate (Wolbach), and they stain with elastin stains, such as fuchsin and safranine (Vogel, Herxheimer and Roth), and especially with the purple component of Mallory's phosphotungstic acid-hematoxylin stain (Wolbach).

Wolbach noted that some parts of the inclusions stained differently from others. With phosphotungstic acid-hematoxylin, a central body and the spines stained dark blue, and the material which surrounded the central body and from which the spines radiated stained pale brown. The individual spines consisted of a peripheral purple material and a core continuous with the material surrounding the central body. The tips of

the spines stained solidly. Strong reagents decolorized the central body from the periphery inward; the spines were less resistant to decolorization. The spines and central body stained deeply by the Gram method; the intervening material, with the counterstain. Herxheimer and Roth, noting that the central granule of some radial structures stained with the Fischler and the Lorraine-Smith methods, concluded that this portion was lipin.

The results of such contrasting stains demonstrating affinities for both dyes or at least a pronounced affinity of certain parts for one dye over the other, and, to some extent, the decolorization tests, suggest that the inclusion material is a complex of something with crystalline form and a substance or substances which impart staining affinities to the periphery and central mass, and that decolorizing agents such as acid alcohol and potassium permanganate followed by oxalic acid may remove the substance or substances responsible for the staining qualities but leave the inclusion largely intact.

EXPERIMENTS

Many of the microchemical tests and staining properties of the radial inclusions summarized in these paragraphs were verified with sections of the spleen tissues in the first postmortem examination described. The inclusions stained dark purple with phosphotungstic acid-hematoxylin. They did not stain with sudan III (formaldehyde-fixed tissues); they were not blackened by the Bielschowsky stain, and they did not react with the microchemical tests for iron, calcium or uric acid. The inclusions and the cell structures of the tissues lost their affinity for the purple component of the phosphotungstic acid-hematoxylin stain by leaching with normal sodium hydroxide-solution. Their shape remained intact. This suggests that the inclusions are impregnated with a substance having a marked affinity for the purple component of the stain which is not necessarily the inclusion material itself. Wolbach noted that a small spherule in the center, the edges and tips of the spines stained purple; the body portions, pale brown.

The configuration of the inclusions in the giant cells is crystalline. Goldmann, who wrote the original description, considered them crystalline fat; Vogel and Hummel believed them to be an undetermined crystalline substance. If the radial structures are crystals, and such an inference is reasonable, the crystalloid came into the tissues in solution and separated according to laws [20] governing crystallization. These state

20. Walker, James: Introduction to Physical Chemistry, New York, The Macmillan Company, 1922. Shade, H.: München. med. Wchnschr. **58**:723, 1911. Wells, H. G.: Chemical Pathology, ed. 4, Philadelphia, W. B. Saunders Company, 1920, p. 452.

that the solubility of substances in solution varies with the temperature, and that at a given temperature the solvent may be unsaturated, saturated or supersaturated. An unsaturated solvent becomes saturated or supersaturated at a definite temperature by solution of more of the substance or by concentration of the solvent volume. From supersaturated solutions the solid separates around micellae, and if a crystalloid, it is in crystals. The size and number of these depend on the speed of separation; some are single, and others are joined in a compact crystalline mass.

Solids colloidally distributed in aqueous systems separate in laminae on the inner surfaces of containers or in concentric layers about floating micellae. Each layer of the solid colloid corresponds usually to a phase of separation. Laminated concretions form in aqueous systems even when a crystalloid as well as a colloid separates from solution, and the colloid framework is impregnated so intimately with the crystalloid that microscopically a distinction of structure is impossible. Fibrin dissolved in the minimal quantities of from 0.07 to 0.1 per cent confers the laminated structure to a concretion.

The conclusion that the crystalline inclusions of the giant cells separated from some supersaturated fluid in the tissues was derived from these laws governing crystallization. The fluid reached its state of supersaturation in the body by concentration of the solvent. Since laminated concretions form in the presence of dissolved protein, the original solvent of these radial structures did not contain appreciable amounts of colloid, and consequently the system in which the crystals formed was not aqueous. The deposits of urates in cartilage with gout may seem to contradict this statement, but the colloid content of these tissues may be small, and urates notably separate amorphous and gradually change into the less soluble crystalline form.

These theoretical conclusions were not correlated as quickly as they are here stated. Analyses of the spleen in the first postmortem examination disclosed no excess of inorganic constituents and purine compounds, and the concretions of the urinary bladder and kidney were mixtures of calcium oxalate, carbonate and phosphate. Finally, in digesting 19 Gm. of moist spleen tissue for analysis, a considerable quantity of lipoid material was observed on the surface of the nitric acid digestion mixture. A quantity of lipin, solid at room temperature, was extracted with chloroform. Chloroform extracts of other hydrolized spleen tissues contained much more of this tan-yellow, greasy lipin material. It had an odor resembling hydrous wool fat, did not react with the acetic anhydride-sulphuric acid test for cholesterol and, microscopically, contained rosette-shaped crystals and needles resembling stearin and palmitin, as well as the radial inclusions of the giant cells. Some unsaturated fatty acid was demonstrated by the bromine test. The

melting point of these fractions ranged between 65 and 75 C. A lead salt insoluble in ether melted at about 147 C., but this result was not confirmed because of the small amount of material available for analysis. These lipins were recovered from many parts of the spleen, not alone from tissues with fibrous nodules. The results of these examinations and the determinations of the melting point suggested that the inclusions were probably stearin, palmitin or derivative compounds, and that these substances were responsible for the lesions in the tissues.

When neutral fats are lodged extracellularly in the body following trauma or necrosis of fatty tissues or injection, their orderly disposal is disturbed. Berner [21] observed that subcutaneous fat about abscesses had retrogressive changes similar to those occurring with fat necrosis. First acidophilic granules appeared in the fat cells, then rosettes of crystals and, finally, masses of calcium soaps and débris. Heyde [22] and Verebély [23] noted crystalline structures in the reparative changes of injured fat tissue, and chronic granulation tissues containing vacuolated phagocytes and foreign body giant cells. Abrikossoff [24] mentioned among the ultimate results of the liberation of fat in tissues the formation of granulation tissue with tubercle-like structures and, eventually, a scar. Embolic fat in the circulation may cause similar tissue reactions. Wuttig [25] injected rabbit fat (melting point, 41 C.) into the liver tributaries of the portal vein. After from six to ten days he noted very little tissue reaction in the capillaries but a marked proliferation of the endothelium in the larger branches, and giant cells. The large bundles of star-shaped and feathery needles, soluble in alcohol, were considered stearin and palmitin. Smith and White [26] observed in fatty cells, especially of the liver, crystalline forms commonly designated as margarine, margaric acid, stearin or stearic acid, which were neutral fats and not fatty acids.

According to Corper and Freed, [27] mild proliferative changes occur in the lungs of rabbits following intratracheal injections of olive oil and liquid petrolatum. Laughlen [28] noted similar changes in the lungs of children not only when oil was introduced into the trachea but also when it was sprayed in sufficient quantities into the nose and throat. Pinkerton [29] reported more extensively the effect of oils and fats in the lungs of children. He [30] later studied experimentally in dogs and rabbits

21. Berner, O.: Virchows Arch. f. path. Anat. **193**:510, 1908.
22. Heyde, M.: Deutsche Ztschr. f. Chir. **109**:500, 1911.
23. Verebély, T.: Beitr. z. klin. Chir. **54**:320, 1907.
24. Abrikossoff, A.: Centralbl. f. allg. Path. u. path. Anat. **45**:396, 1929.
25. Wuttig, Hans: Beitr. z. path. Anat. u. z. allg. Path. **37**:378, 1905.
26. Smith, J. L., and White, C. P.: J. Path. & Bact. **12**:126, 1907.
27. Corper, H. J., and Freed, Harold: J. A. M. A. **79**:1739, 1922.
28. Laughlen, G. F.: Am. J. Path. **1**:407, 1925.
29. Pinkerton, Henry: Am. J. Dis. Child. **33**:259, 1927.
30. Pinkerton, Henry: Arch. Path. **5**:380, 1928.

certain phases of this problem. He found that oily substances were transported from the lungs into the parabronchial lymph nodes and into the spleen. In the various tissues they stimulated proliferative reactions. Pinkerton concluded that the original fatty acid content of a fat and the speed with which free fatty acids are formed by hydrolysis determine the reactive response of the tissues. The simple vegetable oils are bland, he stated, because they have no free fatty acids and the tissues have no specific lipases. The animal fats are irritants because moisture, warmth and enzymes in the tissues hydrolyze the fat and liberate the fatty acids. Many authors have mentioned the similarity between the lesions of tuberculosis and those caused by fats in the tissues. Sabin, Doan and Forkner [31] produced lesions in rabbits with the acetone-insoluble lipins of human tubercle bacilli, which closely simulated those produced by actual infection with Bacillus tuberculosis. Saturated fatty acids were important constituents of the lipin fractions. Various authors have emphasized the similarity between the lesions of tuberculosis and those containing giant cells with radial inclusions.

Attempts to produce lesions in rabbits comparable to those in human tissues by intravenous injections of olive oil, extracted human and mutton fats, olive oil containing additional quantities of palmitin or stearin, oleic acid with palmitin or stearin, calcium or magnesium palmitates and stearates dissolved in oleic acid or in olive oil with sufficient quantities of palmitic or stearic acid to effect solution were unsuccessful. These results indicated that some chemical changes or additional substances were necessary in order that such lipin material should stimulate the growth of chronic granulation tissues. The giant cells with the inclusions described in the published reports and in the tissues of this account were observed frequently in granulation tissues having clefts such as remain where cholesterol has been dissolved. Olein, the important fat of human tissues, liquid at body temperature, holds in solution palmitin, stearin, cholesterol and other lipin substances. When cholesterol is deposited in tissues, it stimulates foreign body granulation tissues,[32] and tissue lipoids containing cholesterol [33] produce lesions resembling tubercles. The presence of cholesterol or a similar substance in the lipoid material seemed necessary, therefore, in order to produce characteristic granulation tissue lesions. A lipin mixture of fat extracted from human omentum with heat (90 C.), fortified with cholesterol from human gallstones and palmitin or stearin,[34] when injected intravenously into

31. Sabin, Florence R.; Doan, C. A., and Forkner, C. E.: Am. Rev. Tuberc. **21**:290, 1930.

32. Le Count, E. R.: J. M. Research **7**:166, 1902.

33. Simonds, J. P.: Am. J. Path. **3**:13, 1927.

34. Some of these mixtures contained a small quantity of suspended finely powdered calcium carbonate.

rabbits stimulated in the lungs foci of chronic granulation tissue with giant cells resembling closely the lesions found in human tissues. These mixtures were supersaturated with palmitin or stearin when injected, so that at the body temperature of the rabbit a certain amount crystallized in rosettes. Animals given injections were killed from two to three weeks after the first one. The lesions in the lungs had a fibroblastic stroma, epithelioid cells, lymphocytes and one or more foreign body giant cells (fig. 3). Large lesions had many giant cells and clefts such as remain where cholesterol has been dissolved. Certain giant cells

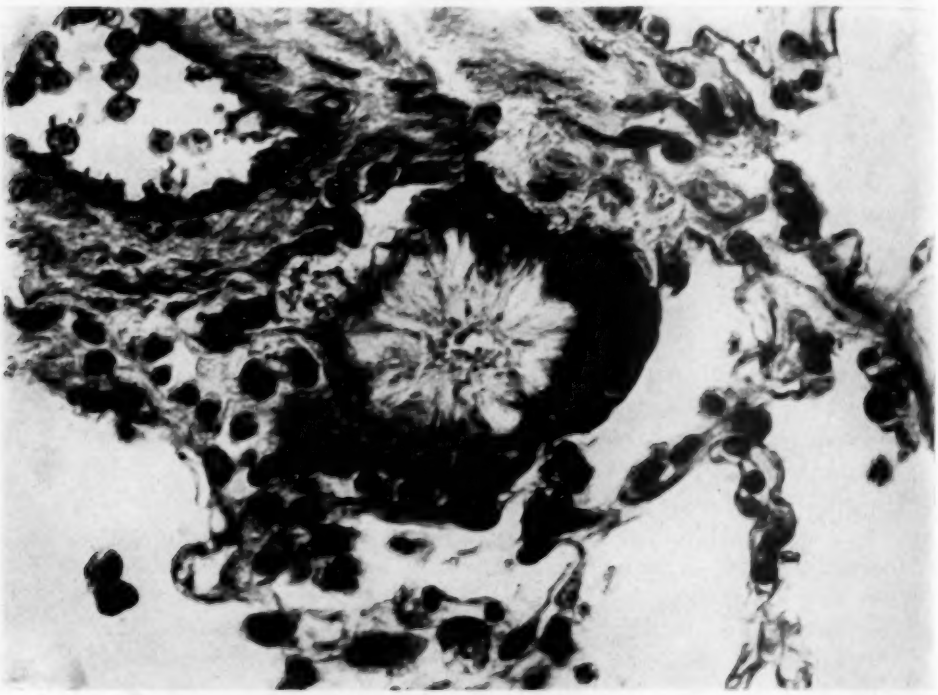

Fig. 3.—Giant cell produced in the lungs of a rabbit with human fat containing cholesterol and stearin. The crystallization factors in this artificial system are comparable only to those occurring in human tissues with disease. Note that much of the lipin crystal has become insoluble in fat solvents. The margins and tips of the spines have the staining qualities of elastin material. Phosphotungstic acid-hematoxylin stain; magnification, × 1,065.

contained small crystals and vacuoles. Some were only vacuolated; others had engulfed or encompassed large rosette-shaped crystals, and many encircled droplets of lipin material in various stages of absorption. Where absorption of the lipoid substance had progressed considerably, a clear part of the cell contained a granular material and a fairly complete radial structure. Cells with partially absorbed globules had a large

vacuole and peripheral portions of the rosette projecting radially into the cytoplasm around the vacuole. The crystals, at least those retained in the tissues sufficiently long, had become insoluble by their contact with the tissues. In sections stained with phosphotungstic acid-hematoxylin, the delicate spines were purple throughout; the coarser had a red-brown core and a thin purple margin.

These experiments, of course, indicate simply how a lipin mixture containing cholesterol may stimulate tubercle-like lesions with giant cells in tissues and that crystals of palmitin or stearin separating from such mixtures may become insoluble rosette inclusions in giant cells. Perhaps other lipin mixtures containing substances having the properties of cholesterol can stimulate similar granulation tissue reactions. The chemical tests of the lipin material recovered from the human spleen failed to demonstrate cholesterol, but these fractions were obtained from spleen tissues mainly between, and not in, the fibrous nodules. Presumably, additions of cholesterol-like substances from necrotic tissues to the lipins or chemical alterations of the lipin material occurred focally and initiated the granulation tissue reaction, or such mixtures were deposited focally and stimulated the granulation tissues wherever they lodged. The splenic pulp of rabbits that received injections of human fat containing cholesterol and palmitin had extensive regions of inflammatory tissues but no giant cells—changes unlike those of the lungs, where much of the dissolved cholesterol and saturated fat separated from solution.

The evidence favoring the conclusion that the radial structures of giant cells in systemic and focal masses of chronic granulation tissues are originally crystalline fats solid at body temperature, such as palmitin and stearin, separating from oily mixtures containing cholesterol or cholesterol-like substances, is derived from: (1) the circumstance that the lesions with these giant cells, according to published reports and in the investigated tissues, have been associated with fat tissues or have contained clefts such as generally are recognized as remaining in tissues where cholesterol has been dissolved; (2) the chemical analysis of spleen tissues with such lesions whereby large amounts of lipin of the character of stearin, palmitin or mixtures were demonstrated and which on solidification formed rosette-shaped crystals, and (3) the experiments demonstrating that lipin mixtures of cholesterol with a high content of palmitin stimulate in the lungs of rabbits tubercle-like lesions with giant cells having radial inclusions and comparable to those seen in human tissues. The crystals so lodged in the tissues gradually became insoluble in fat solvents and had, in sections stained with phosphotungstic acid-hematoxylin, a purple periphery and a red-brown center. These staining qualities favor the conclusion that substances from the tissues confer

the elastin-like staining qualities to the crystalline fat, but that other changes of the crystalloid substances render them insoluble in the usual fat solvents.

CONCLUSIONS

The radial inclusions of giant cells observed in tubercle-like granulation tissues are crystalline forms of fats solid at body temperature, such as palmitin or stearin, separated from an oil system containing cholesterol or substances with the physical properties of cholesterol.

The formation of these crystals in a liquid fat system is according to the usual laws governing crystallization, and the factors accomplishing supersaturation of the system are mainly the abstraction of the liquid portion faster than the combustion of the dissolved solid fat.

Certain chemical changes take place in the composition of the crystals in the tissues so that they become insoluble in fat solvents. Further changes or additions in the tissues produce the elastin-staining qualities.

An embolic dissemination through the blood and lymph channels distributes the lipoid material into the liver, spleen, lungs and lymph nodes—visceral tissues commonly the site of systemic lesions. Lipoid, insufflated or aspirated into the respiratory passage, may initiate lesions along the bronchi and bronchioles.

BASE-PROTEIN-ACID COMPOUNDS

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AND
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CINCINNATI

The physicochemical constitution of naturally occurring proteins, such as the protoplasm of animals or plants, eggwhite, blood plasma or milk plasma, continues to be a matter of debate, even though the purely chemical attack on the problem was initiated in the sixties of the last century and the physicochemical attack but a few years later. The opinion of the majority today still holds that these natively occurring materials are mere mixtures of two or more proteins suspended in water. Since their properties when chemically pure are in no wise those of these same proteins when encountered naturally, the effects of the inorganic acids, bases and salts constantly present in living matter or its juices have been called on to explain the difference. The accepted point of view here again is that these "electrolytes" are materials that are merely "dissolved" (in dilute solution) in the water of protoplasm and thus "influence" the behavior of the mixed proteins.

Against this point of view which, briefly stated, holds that living matter (such as any of the meats) is an aggregation of droplets composed essentially of a solution of salts in which protein is suspended, stands the simple fact that the tissues of plants and animals will not "mix" with more of their solvent (water). Even egg white or blood plasma will not do so without chemical decomposition (increase in alkalinity, precipitation of globulin). This by itself proves that the high percentage of water found in living matter is not in the same form as ordinary water; in other words, it cannot be "free" but must be held in a chemically combined form.[1] Protoplasm is therefore a "hydrate." But the salts found in protoplasm (commonly obtained by ashing it) cannot easily be leached out, which proves that they too are not merely "dissolved" but held in combination. The necessary conclusion is therefore that the essence of living matter is a protein (or several proteins in combination or mixture) to which the inorganic radicals of the salts are tied chemically in the form base-protein-acid and not in the form protein-salt.

Comparative colloid-chemical studies of tissues, on the one hand, and of various proteins, on the other, confirm this point of view. Thus, pure

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1. Fischer, Martin H.: Œdema, New York, John Wiley & Sons, 1910, p. 184.

proteins (like the globulins) take up very little water, but they swell enormously as soon as acid or alkali is combined with them. The tissues of the body, however, are more than either basic proteinates or acid proteinates. *They are both in one.* This is proved by the physiologic, pharmacologic or toxicologic action of the various salts on them. All neutral salts dehydrate the living mass but, at the same concentration, very unequally. Thus the chlorides of potassium, magnesium, calcium and mercury dehydrate increasingly in the order named (and so reduce the body weight or an edematous condition and act as hydrogogic cathartics, diuretics or sudorifics in the order named), while the acid radicals, united to any base, dehydrate increasingly in the following order: chloride, acetate, sulphate and phosphate (exhibiting a like order of action when employed therapeutically).[2] The physiologic action of any salt comes, therefore, to be compounded of the action of its constituent radicals (which makes magnesium sulphate a more powerful cathartic or diuretic than sodium chloride and makes a mercury salt the most powerful of all). The fact that *both* radicals are physiologically active is proof that *both* acid and basic radicals appear in the native protoplasm.

In colloid chemistry pure basic proteinates, which are hydrated in the order of the base series here outlined, and pure acid proteinates, which are hydrated according to the acid series, have been prepared. But base-protein-acid compounds that are analogous to those existent in the living mass have not yet been produced. These paragraphs describe how they may be.

THE PRODUCTION OF BASE-PROTEIN-ACID COMPOUNDS

The production of a basic proteinate or an acid proteinate is simple. Mere addition of any hydroxide or acid to a protein will yield some of the compound, but to have the reaction complete *it must be carried out in the absence of free water.* The problem is analogous to the production of a soap from a fatty acid and an alkali. Here, too, only when carried out in concentrated form (in other words, without the presence of free water) is this reaction complete. *Utilization of the same principle on a protein allows not only a base or an acid but both to be tied to the protein.*

Under ordinary circumstances sodium caseinate or casein chloride on the addition of an acid or an alkali reverts to its pure and anhydrous form (it is said that the acid or alkali reacts with the base or acid in the caseinate, frees the casein and allows it to fall out as a precipitate).

2. Fischer, Martin H., and Sykes, Anne: Science **37**:845, 1913; Kolloid-Ztschr. **13**:112, 1903; Œdema and Nephritis, ed. 2, New York, John Wiley & Sons, 1915, p. 295.

If the same additions are made, but in a reaction mixture in which all the water is held in hydrate form (in practical terms, and for casein this means in any amount below 80 per cent of the total mixture), the acid or alkali does *not* crack off the contained base or acid, but *both* combine with the protein nucleus.

The eighty per cent of water in these systems (not free but held in hydrate form) is a first value of physiologic significance to be remembered in these studies. It needs to be compared with the normal percentage of water discoverable in the composition of any of the ordinary tissues. Human blood, for example, carries only about 80 per cent of water, heart muscle 79 per cent, skeletal muscle 75 per cent, the brain 74 per cent, the skin 72 per cent, the liver 68 per cent and the bones 62 per cent.[3]

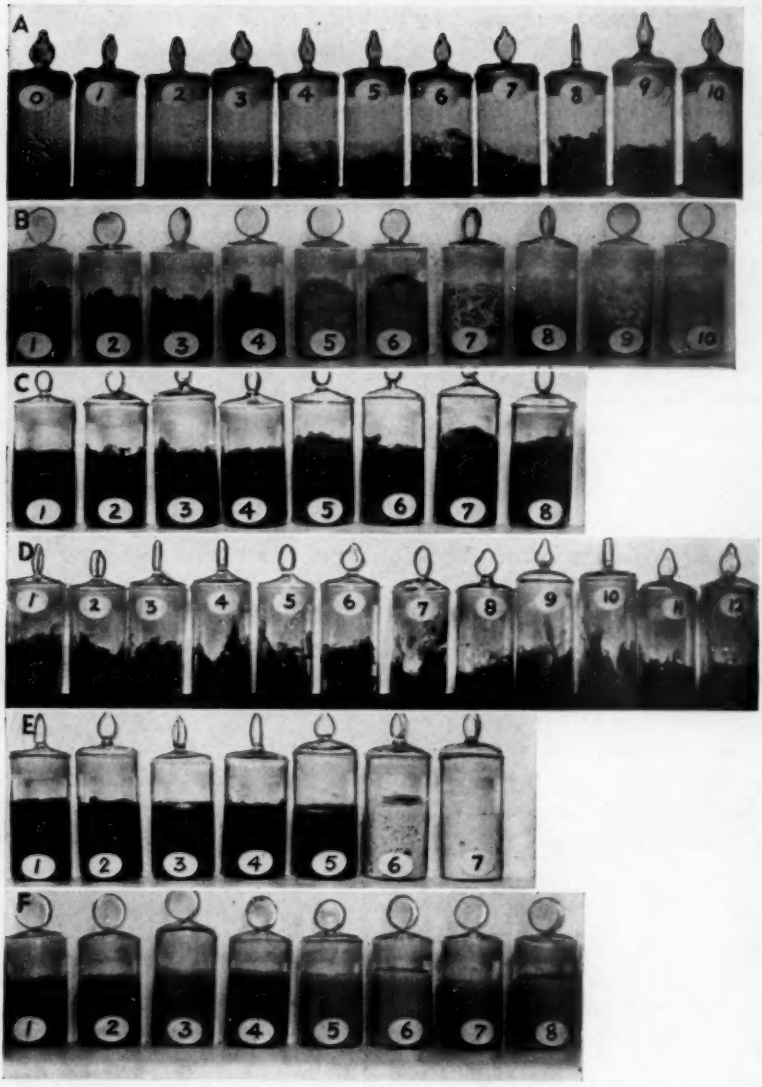
In the accompanying figure, *A* illustrates the point that acid fails to knock alkali off a basic proteinate, provided the mixture is concentrated and anhydrous. Vessel *0* contains a mixture of pure sodium caseinate and water made by adding to 50 Gm. of a highly purified casein 20 cc. of water and 80 cc. of half-normal sodium hydroxide.[4] The product is a faintly straw-colored transparent gel. The succeeding vessels contain an identical mixture, but there has been added to them strong hydrochloric acid in such quantity that the final mixture (vessel *10*) contains just enough for complete neutralization of the base that was originally added to the casein. The intermediate tubes contain increasing amounts (in steps of 10 per cent) of the acid. It will be observed that over 70 per cent of the acid and not exceeding 80 per cent may be added before any change in the gel is perceptible.[5] Beyond this point all the water is still bound, but the gels are drier and stiffer. When stirred they tend to fracture like crystals and so appear whiter. Their physical characteristics change, in other words, from those of egg white to those of a torn liver or kidney.

In the figure, *B* proves that only as free water is absent from the reaction mixture does the added acid join to the protein (and not to its contained alkali). Vessel *1* contains potassium caseinate (50 Gm. of casein and 80 cc. of half-normal potassium hydroxide) to which was added enough strong hydrochloric acid to neutralize completely the fixed

3. Such analyses may be found in Vierordt, Hermann: Daten und Tabellen, ed. 3, Jena, Gustav Fischer, 1906, p. 377.

4. This is the neutralization equivalent of casein according to T. B. Robertson.

5. Workers who wish to repeat these experiments need to remember that these colloid reactions take time. It requires about twenty-four hours for an acid or an alkali to combine with casein and to have the whole system come to equilibrium so far as the absorption of water is concerned. The same is true when acid is added to a basic caseinate, which should be done in fractions and slowly, with due care to the securing of an immediate and uniform mixture.



A, standard sodium caseinate to which increasing increments of hydrochloric acid have been added up to the point of complete neutralization of the base. *B*, standard potassium caseinate to which increasing increments of water have been added and then hydrochloric acid to the point of complete neutralization. Casein is definitely precipitated only in vessels *8*, *9* and *10*. *C*, standard potassium caseinate to which chemical equivalents of different acids (in the following order: phosphoric, citric, acetic, lactic, hydrochloric, tartaric, hydrobromic and sulphuric acid) have been added. *D*, standard sodium caseinate to which chemical equivalents of different fatty acids (of the acetic series and from formic through stearic) have been added. *E*, a series of different basic caseinates to which chemical equivalents of phosphoric acid have been added. *F*, a series of different acid caseinates (in the following order: phosphate, citrate, acetate, lactate, chloride, tartrate, bromide and sulphate) to which chemical equivalents of potassium hydroxide have been added.

base. But no externally visible change has occurred in the gel. The remaining vessels contain the same potassium caseinate (with its 80 cc. of water), but before the necessary equivalent of acid was added, increasing amounts of water (in steps of 40 cc.) were poured into them. Failure of the acid to combine completely with the protein (combining instead with the potassium of the caseinate) is apparent in vessel *5*, in other words, when the original amount of potassium caseinate has been mixed with 240 cc. of water. In percentage composition this represents progression from 60 per cent of water in vessel *1* to 83 per cent in vessel *5*. Beyond this point (it should again be noted that it already lies well above the average value of the water content of all solid animal tissues) the action of the acid becomes increasingly that of neutralization of the fixed alkali in the caseinate (thus leading to a precipitation of the neutral casein and dehydration of the entire system). In vessel *10* (which contains 440 cc. of water) separation of the neutral casein is complete.

C shows that every acid acts like hydrochloric acid on a given basic caseinate. Vessels *1* to *8* contain equal amounts of potassium caseinate (prepared by adding 200 cc. of fifth-normal potassium hydroxide to 50 Gm. of casein). There has been introduced into the successive vessels strong phosphoric, citric, acetic, lactic, hydrochloric, tartaric, hydrobromic and sulphuric acid in the order named and in an amount to neutralize completely the potassium of the system. There is no difference in the several systems except as the first may be said to be the most homogeneous and the last, the least liquid. "Precipitation" of neutral casein has not occurred anywhere.

D in essence parallels *C*. The vessels show that practically identical gels are obtained, so far as physical appearance is concerned, when the several members of the acetic series of fatty acids are added to the standard sodium caseinate prepared as in *A*. Formic, acetic, propionic, butyric, valeric, caproic, caprylic, capric, lauric, myristic, palmitic and stearic acids have been added in chemically equivalent amounts to vessels *1* to *12*. In vessels *10* to *12* the reaction mixtures were warmed.

The findings described are of importance in connection with the problem of the absorption of fatty acid by any tissue or, specifically, by the intestinal tract. The solubility of these acids in water falls almost to zero when valeric acid is passed. To explain the absorbability of the higher members of the series, their combination with bile acids (to yield more "soluble" compounds) has recently been urged.[6] *D* shows that they are capable of direct absorption, through combination with protein, even up to stearic acid, although as the series is ascended the process takes longer and may be less complete.

6. Tashiro, Shiro: Personal communication to the authors in 1935.

The effects of adding a given acid (phosphoric) to a series of different basic caseinates (all perpared by mixing together 50 Gm. of casein and 200 cc. of water and the necessary weight of base to yield a fifth-normal mixture) is shown in *E*. The gels ammonium casein phosphate, potassium casein phosphate and so on through sodium, lithium and magnesium (vessels *1* to *5*) require no comment. Those of calcium casein phosphate (vessel *6*) and plumbic casein phosphate do require comment, for while all continued to hold the water of their systems, they were less transparent. We take this to mark aggregation of the particles of protein into larger masses and as evidence of their lowered capacity for hydration. The situation has its parallel in the behavior of the corresponding metal soaps, though it remains a question in these experiments if, on allowing casein to stand with calcium hydroxide or lead oxide for several days, all the casein and base capable of combination have really reacted.

If the alkali and acid added to the reaction mixtures here described (containing about 80 per cent of water) are recalculated as their "salt" content, it amounts to 1.5 per cent. This is again a value of physiologic significance, for it lies well above the normal salt content of all fresh tissues or body fluids. The ash from spleen, for example, represents only 1.5 per cent; from brain, 1.41 per cent; from lung, 1.16 per cent; from intestinal canal, 1.07 per cent; from heart muscle, 1.06 per cent; from blood, 0.85 per cent; from kidneys, 0.8 per cent, and from skin, 0.7 per cent.[3] But a protein hydrate that is first neutralized to its capacity with alkali shows no visible change on the addition of acid until more than 70 per cent of the neutralization value for the alkali is exceeded. This relation, too, is biologically significant because it is identical with the proportion of total base to total acid discoverable in the salts analyzed out of milk, blood plasma or any solid tissue. For instance, if the total base is taken as unity, the total acids of milk (with the phosphorus and sulphur of the protein and lipins included) constitute only 90 per cent of this value;[7] for whole blood the value stands at 58 per cent;[8] in the case of muscle, at 78 per cent.[9]

Being in essence linkages of amino-acids, it is not unnatural that proteins should combine with bases. But they are amphoteric and combine also with acids, even though the total combining value for them is lower (by about 30 per cent). Combination with acid also takes more time. We were interested in discovering whether acid caseinates (in the absence of free water) would combine with alkalis and so yield

7. Lincoln, Azariah Thomas, and Walton, James Henri: Quantitative Chemical Analysis, New York, The Macmillan Company, 1914, p. 88.

8. Vierordt,[3] p. 200.

9. Vierordt,[3] p. 426.

the triple compounds already described. This they do, as evidenced in *F*, even though the gels produced are not as homogeneous as when the reaction is carried out in the opposite direction. In all these vessels the acid caseinates (in the following order: casein phosphate, citrate, acetate, lactate, chloride, tartrate, bromide and sulphate) were first made by adding to 50 Gm. of casein 200 cc. of the appropriate two-twenty-fifths-normal acid. (This amounts to only two fifths of the acid used for complete neutralization of the base when the basic caseinates were used as starting materials, purposely kept low in order not to exceed the neutralization capacity for acids of the casein itself.) After the addition of dry potassium hydroxide to the several acid caseinates (just sufficient to neutralize the acid), as the vessels show, no precipitation of the casein occurred.

We have also tried to produce these triple compounds directly by triturating dry salt into casein in the presence of minimal quantities of water or by allowing the pure casein to stand for long periods in contact with concentrated salt solution. In the instance of neutral salts (like sodium chloride) no change is noticeable in the hydration of the casein, but when salts that are definitely alkaline (like the potassium soaps) or acid (like the chlorides of weaker bases) are employed, partial union does take place, and (nonhomogeneous) gels are frequently obtained.

SUMMARY

After citation of some of the evidence which indicates that the "native" proteins are base-protein-acid compounds, a method is described which allows of their production artificially. The end is accomplished by working with reaction mixtures containing no free water and by adding to any protein (casein was used in the illustration) first a base and then an acid or vice versa. The allowable limit of water content for these systems, in which alone such reaction was possible, was found to lie well above the normal water content of living tissue; while the amount of "salt" that could thus be bound to a protein, as well as the percentage relation of the base to the acid in such "salt," proved identical with biologic values.

MECHANISM OF PATHOLOGIC CALCIFICATION

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No attempt will be made to review the literature on various phases of calcification, because such reviews have been made recently by Ham,[1] Thomson and Collip,[2] Kay [3] and Barr.[4] Several theories have been advanced in attempts to explain the mechanism of calcification of the soft tissues of the body. It is known that alkalinity favors the precipitation of calcium salts in vitro, and Hofmeister [5] suggested that the calcification of the arteries might be due to the decreased carbon-dioxide tension of arterial blood with resulting increase in alkalinity. He also suggested that the tendency of the alveoli of the lungs, uriniferous tubules and gastric glands to calcify was due to the alkalinity of these structures brought about by the elimination of acids at these sites. Kleinmann [6] found that dead tissues and necrotic areas were alkaline in reaction, and he attributed their tendency to calcify to this fact. It is recognized that fatty degeneration frequently precedes calcification of the soft tissues, and according to Klotz [7] the formation of calcium soaps, which are subsequently transformed into calcium phosphate and calcium carbonate, constitutes the mechanism of calcification. Wells [8] has presented evidence which would seem to indicate that the theory of Klotz regarding calcification is untenable. According to Robison,[9] normal ossification is brought about by the enzyme phosphatase. It is recognized that the presence of vitamin D is necessary for normal ossifi-

From the Department of Physiology, University of Illinois.

1. Ham, A. W.: Arch. Path. **14**:613, 1932.
2. Thomson, D. L., and Collip, J. B.: Physiol. Rev. **12**:309, 1932.
3. Kay, H. D.: Physiol. Rev. **12**:384, 1932.
4. Barr, D. P.: Physiol. Rev. **12**:593, 1932.
5. Hofmeister, F.: Ergebn. d. Physiol. **10**:429, 1910.
6. Kleinmann, H.: Biochem. Ztschr. **196**:161, 1928.
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9. Robison, R.: Biochem. J. **17**:286, 1923.

cation, and evidence has been presented to show that large doses of viosterol, as well as of the internal secretion parathormone, may bring about calcification of the soft tissues.[10] It is known that the deposition of calcium salts begins in the intima of the arteries, and there is considerable evidence that this is initiated by injury resulting, for example, from an infection such as syphilis, the injection of certain poisonous chemicals, the stress and strain of modern life or excessive physical work.

Active, injured and dying tissues are electronegative to inactive, uninjured and sound tissues. The injured end of a muscle, for instance, is electronegative to the sound surface, and the contracted portion of a muscle is electronegative to the relaxed portion. The contracted part of the heart is electronegative to the uncontracted part, and this fact renders the making of the electrocardiogram possible. It was while studying these differences of potential in the animal and measuring the action and demarcation currents that it occurred to us that herein might be found an explanation for calcification of the soft tissues, and the following investigation was accordingly carried out.

EXPERIMENTS

With the use of ammonium molybdate paper the injured or cut ends of gastrocnemius muscles of frogs were tested for phosphate, and it was found to be present in definitely larger quantities on the cut end than on the uninjured surfaces. This suggested to us that the electronegativity of the injured portions of these muscles might be caused by the negatively charged phosphate ions, and the following experiments were carried out to determine whether this was true.

The gastrocnemius muscles of frogs were removed, skinned and cut transversely near one end. The cut end was placed against one nonpolarizable boot electrode and the sound surface against the other electrode, as shown in the insert in figure 1. Both boot electrodes were kept moist with a physiologic solution of sodium chloride. By means of wires a delicate micro-ammeter was connected with the boot electrodes and the demarcation current measured in micro-amperes. For the muscles of these medium-sized frogs the demarcation current was found to be of the magnitude of from 2 to 4 micro-amperes. It was found that the application of a solution of calcium chloride, as well as of barium chloride, to the cut end of the muscle caused the disappearance of the demarcation current, and the application of a weak solution of phosphoric acid or of disodium phosphate restored the current to its original value. It was also found that if a strip of filter paper or a piece of wire was placed across the electrodes instead of the muscle and if one end of the filter paper or wire was moistened with a solution of phosphoric acid or disodium phosphate and the other end with a

10. Hueper, W.: Arch. Path. **3**:14, 1927.

physiologic solution of sodium chloride, a current was set up similar to the demarcation current of the injured muscle and of about the same magnitude. This current passed from the phosphate to the chloride end of the paper or wire. The application of calcium chloride to the end of the filter paper or wire that was moistened with the phosphate decreased the current in the same way that it decreased the demarcation current when applied to the cut end of the muscle. The current could be restored by moistening the end of the filter paper or wire with phosphoric acid, as had been found to be the case with the muscle.

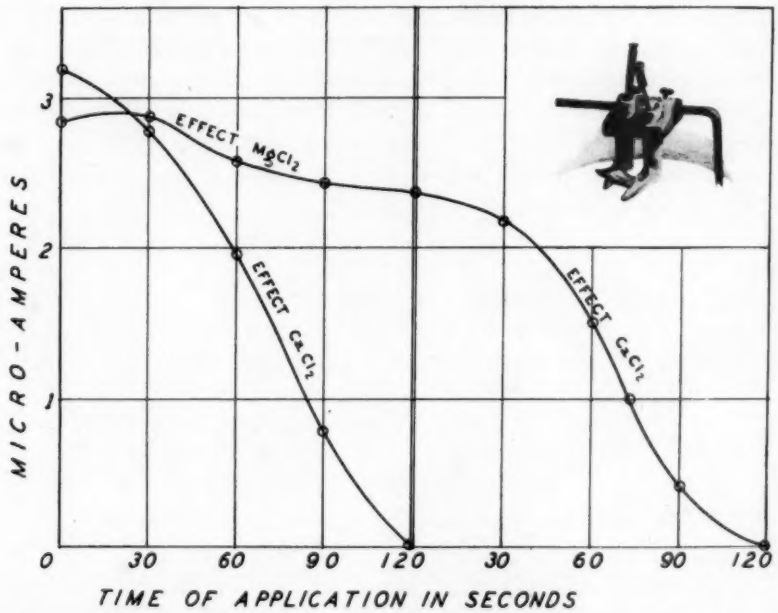

Fig. 1.—The insert shows the injured or electronegative end of a muscle against one boot electrode and the sound or electropositive surface against the other electrode. The curves show that the application of calcium chloride to the injured end of the muscle does away with the demarcation current, while magnesium chloride has only a little effect.

In figure 1 are given curves showing the quantitative effect of application of a twice normal solution of calcium chloride and magnesium chloride on the demarcation current of injured gastrocnemius muscles of frogs. The method of applying the calcium chloride and magnesium chloride was to suspend the muscle and permit its cut end to touch the surface of the solution for thirty second intervals and measure the demarcation current after each application. It will be seen in figure 1 in the curve for the effect of calcium chloride that the demarcation current of the muscle previous to the application of calcium chloride was

3.2 micro-amperes; after thirty seconds of treatment with calcium chloride it was reduced to 2.8 micro-amperes; after the second thirty second application or after sixty seconds of treatment with calcium chloride, to 2 micro-amperes, and after ninety seconds, to 0.75 micro-amperes. After one hundred and twenty seconds the current had disappeared. It may also be seen in figure 1 in the curve for the effect of magnesium chloride that a twice normal solution of magnesium chloride had little effect on the demarcation current. The curve for the effect of magnesium chloride and of calcium chloride shows that after magnesium chloride had been applied to the cut end of the muscle and had produced practically no effect, the application of calcium chloride to the cut end of the same muscle promptly decreased the current and caused it to disappear. It should be mentioned in this connection that barium chloride was found to be as effective as calcium chloride in causing the disappearance of the demarcation current. It is assumed that the positively charged calcium and barium ions did away with the demarcation current by combining with the negatively charged phosphate ions at the cut end of the muscle to form insoluble and non-ionized barium phosphate and calcium phosphate, whereas the application of magnesium chloride formed the more soluble and ionized magnesium phosphate. The preceding observations have been repeated time and again by us, and the experiments have been used by students as a part of routine work in the laboratory.

It is also known that the contracted portion of a muscle is electronegative to the relaxed part, and the observations made on the injured muscle suggest that this electronegativity is probably due also to the negatively charged phosphate ions arising from the hydrolysis of creatine phosphate and adenylpyrophosphate in the contracted part of the muscle. Since massive doses of viosterol and parathormone produce calcification of the soft tissues, the effect of these two substances on the demarcation current was also studied in a manner similar to the preceding, and it was found that the application of these substances to the cut end of the muscle had no effect on the demarcation current.

The demarcation current of the arteries of anesthetized dogs was measured. This was done with the use of a micro-ammeter and platinum electrodes. It was found that when two uninjured parts of the carotid artery were connected with the micro-ammeter practically no current flowed, but when an injured portion, either of the exterior or interior of the artery, was connected to an uninjured part a current flowed from the uninjured to the injured surface, just as was found to be the case with the gastrocnemius muscles. The electronegativity of the injured intima of the artery is attributed to the negatively charged phosphate ions, as in the injured end of the gastrocnemius muscles. and the sub-

sequent calcification of the intima is attributed to the combination of the positively charged calcium ions of the blood with the negatively charged phosphate ions of the injured intima to precipitate the insoluble calcium phosphate and form the plaque.

With advance in age calcium salts are gradually deposited also in the crystalline lens of the eye, resulting in a receding of the near point of distinct vision and loss in the power of accommodation. At 20 years of age a normal person possesses 10 diopters of accommodation, but at the age of 60 all this has been lost, and this loss has occurred at the uniform rate of ¼ diopter per year. So uniformly and constantly does this loss in the power of accommodation occur that the age of a person may be ascertained with a high degree of accuracy by determining the near point of distinct vision. With the accumulation of very large amounts of calcium salts in the crystalline lens of the eye, cataract results.[11]

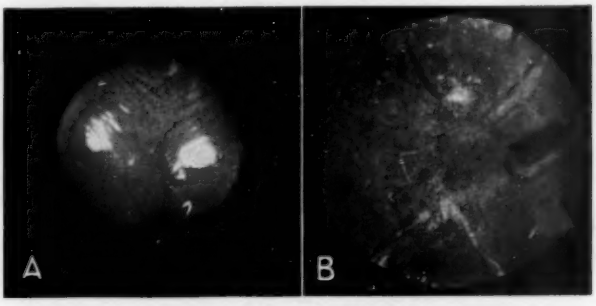

Fig. 2.—Lens 1 (*A*) was immersed in twice normal potassium chloride and lens 2 (*B*) in twice normal calcium chloride. The potassium chloride produced nuclear opacity, leaving the cortex transparent, while the calcium chloride produced cortical opacity without affecting the nucleus.

In figure 2 are shown fresh crystalline ox lenses that had been immersed in twice normal solutions of potassium chloride and calcium chloride. Lens 1 (*A*) was immersed in potassium chloride and lens 2 (*B*) in calcium chloride. It will be seen that potassium chloride produced an opacity of the nucleus of the lens without affecting the transparency of the cortex, and that the calcium produced an opacity of the cortex without affecting the nucleus. This observation suggests that calcium salts may play a rôle in the production of cortical cataract and potassium salts in that of nuclear cataract. It is known that the crystalline lens grows throughout life and that this growth, like that of a cabbage, takes place from the interior. So the oldest portion of the

11. Burge, W. E.: Arch. Ophth. **38**:447, 1909.

lens is the cortex and the youngest the nucleus. Hence, there is a tendency for the older portion of the crystalline lens, the cortex, to combine with calcium salts, comparable with the tendency of the arteries to become calcified with advance in age.

The tender green branches of several different kinds of greenhouse plants were tested to determine whether there was a demarcation current or current of injury in plants similar to that in animals. This was done by placing a tender green branch across the boot electrodes shown in the insert in figure 1, with the cut end of the branch against one electrode and the uninjured surface against the other. When this was done it was found that a current flowed from the uninjured surface of the branch to the injured end, similar to that of the muscle, but the strength of the current was much less than in the muscle. The application of a weak solution of calcium chloride to the injured end of the stem of the plant did away with the demarcation current, just as was found to be the case with the muscle. The inorganic salt deposited in the arteries of animals as they grow older is principally calcium phosphate, whereas calcium oxalate is the salt deposited in plants as they grow older. It is assumed that the negatively charged oxalate ions are responsible for the electronegativity of the injured portion of the plant, just as the negatively charged phosphate ions are responsible for the electronegativity of the injured portion of the muscle. The positively charged calcium ions combine with the negatively charged phosphate ions of the injured muscle and with the negatively charged oxalate ions of the injured plant to precipitate calcium phosphate and calcium oxalate, respectively, and in this way do away with the current of injury or demarcation current and form the calcareous deposit.

SUMMARY

With the use of a micro-ammeter the demarcation current of injured frogs' muscles was measured and found to be of the order of magnitude of from 2 to 4 micro-amperes. A demarcation current in injured branches of greenhouse plants was also observed, but this was much less than in the animal muscles.

The presence of phosphate on the injured portion of the muscle was shown by the use of ammonium molybdate paper. The application of a twice normal solution of calcium chloride or barium chloride to the injured portion of the muscle or of the plant did away with the demarcation current, and a weak solution of phosphoric acid or disodium phosphate restored it.

The electronegativity of the injured portion of a muscle is attributed to the negatively charged phosphate ions, and the disappearance of the current on the application of calcium chloride and barium chloride, to the

combination of the positively charged calcium and barium ions with the negatively charged phosphate ions, to precipitate insoluble calcium and barium phosphate.

The electronegativity of the contracted portion of a muscle is also attributed to the negatively charged phosphate ions, arising probably from the hydrolysis and ionization of creatine phosphate and adenylpyrophosphate in the contracted part of the muscle.

Injury to the intima of the carotid artery of dogs renders the injured portion electronegative to the uninjured portion, and calcification of arteries may result from the combination of the positively charged calcium ions of the blood with the negatively charged phosphate ions at the site of the injured intima to precipitate calcium phosphate.

Potassium chloride produced an opacity of the nucleus of the crystalline lens without affecting the transparency of the cortex, while calcium chloride produced an opacity of the cortex without affecting the nucleus. This observation suggested that calcium salts may play a rôle in the production of cortical cataract, and potassium salts in the production of nuclear cataract.

SUSCEPTIBILITY TO DENTAL CARIES IN THE RAT

V. INFLUENCE OF CALCIUM, PHOSPHORUS, VITAMIN D AND CORN OIL

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In previous reports [1] we have described the production in rats of experimental dental caries and indicated some of the conditions that give rise to the disease or modify its course. We have shown: (*a*) that the lesions in question, identified microscopically in all instances, closely resemble naturally occurring dental caries in man and differ in important respects from lesions described as "macroscopic caries" by many other workers; (*b*) that experimental caries results from feeding diets the major constituent of which is coarsely ground rice or corn, and that the disease does not occur when the cereal is very finely ground; (*c*) that experimental caries may be produced either with diets deficient in minerals, vitamin D and protein or with diets adequate in all nutritional respects, but in the latter case the incidence of experimental caries is lower than in the former, and (*d*) that the relative protective effect against experimental caries produced by adequate diets appears to depend on some one calcifying food or some combination of the calcifying foods, but the precise agency involved could not be determined from the data.

EXPERIMENTS

The experiments reported here were an attempt to define more exactly the food agent or agents responsible for the reduction of experimental caries observed in rats that received diets with adequate calcifying properties. Two series were studied, for each of which the basis of reference was the deficient diet of rice passed through a sieve having 10 meshes per linear inch (2.54 cm.), dextrin and spinach, which was previously employed, to which additions were made individually. The diets used are given in table 1.

In series I, diets 55 and 56 contain calcium and phosphorus in relatively high levels with an adequate ratio (1.5) without cod liver oil and with 2 per cent of cod liver oil. Diet 57 is the basal control. Diet 58 contains rice passed through a 100-mesh sieve in place of that passed through a 10-mesh sieve. In diets 60

This study was conducted in part with the aid of a grant from the Commonwealth Fund for research in dental caries.

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1. (*a*) Rosebury, T.; Karshan, M., and Foley, G.: J. Dent. Research **12**: 464, 1932; (*b*) ibid. **13**:379, 1933; (*c*) J. Am. Dent. A. **21**:1599, 1934.

and 61 calcium is added to make a high calcium-phosphorus ratio (6.9); the latter diet again contains rice passed through a 100-mesh sieve. Diet 63 contains calcium and phosphorus added to overcome the gross deficiency of the basal diet but leaving the ratio low (0.4). Diet 64 contains 7 per cent of wheat gluten as the only addition to the basal formula. In series II, diet 65 is the basal control, and each of the experimental diets is supplemented by a single item. Diets 66 and 67 contain, respectively, 2 and 5 per cent of a dilution of viosterol (250 D) in corn oil adjusted to have the vitamin D potency of cod liver oil;[2] diets 68 and 69, 2 and 5 per cent of cod liver oil, respectively, and diets 70 and 71, 2 and 5 per cent of corn oil, respectively. Diet 72 is the same as the control, but animals in this group were irradiated with an air-cooled quartz mercury vapor arc lamp in

TABLE 1.—*Experimental Diets*

	Percentage												
	Brown Rice, 10 Mesh	Brown Rice, 100 Mesh†	White-Potato Dextrin	Calcium Carbonate	Potassium Phosphate (KH_2PO_4)	Cod Liver Oil (Mead's)	Viosterol in Corn Oil‡	Corn Oil (Mazola)	Wheat Gluten	Spinach Leaves#	Calcium Content§	Phosphorus Content	Calcium:Phosphorus Ratio
Series I													
Diet 55	81	..	8	6	5	..	..	..	..	..	2.46	1.61	1.53
Diet 56	79	..	8	6	5	2	..	..	..	..	2.43	1.61	1.51
Diet 57*	92	..	8	..	..	..	..	..	..	..	0.08	0.25	0.12
Diet 58	..	92	8	..	..	..	..	..	..	..	0.08	0.25	0.12
Diet 60	88	..	8	4	..	..	..	..	..	..	1.63	0.24	6.88
Diet 61	..	88	8	4	..	..	..	..	..	..	1.63	0.24	6.88
Diet 63	88	..	8	1	3	..	..	..	..	..	0.43	0.92	0.46
Diet 64	85	..	8	..	..	..	..	..	7	..	0.08	0.24	0.14
Series II													
Diet 65*	92	..	8	..	..	..	..	..	..	..	0.08	0.25	0.12
Diet 66	90	..	8	..	..	..	2	..	..	..	0.08	0.24	0.12
Diet 67	87	..	8	..	..	..	5	..	..	..	0.08	0.24	0.12
Diet 68	90	..	8	..	..	2	..	..	..	..	0.08	0.24	0.12
Diet 69	87	..	8	..	..	5	..	..	..	..	0.08	0.24	0.12
Diet 70	90	..	8	..	..	..	..	2	..	..	0.08	0.24	0.12
Diet 71	87	..	8	..	..	..	..	5	..	..	0.08	0.24	0.12
Diet 72‖	92	..	8	..	..	..	..	..	..	..	0.08	0.24	0.12

* Control.
† Ground repeatedly until the entire product passed through the 100-mesh sieve.
‡ Viosterol 250 D (Mead's), 0.4 per cent in corn oil (dilution = 1D).
§ See Rosebury, Karshan and Foley,[1e] table 8.
# 5 Gm. per rat per day in all.
‖ Rats in this group were irradiated with a quartz mercury vapor arc lamp for twenty minutes at 2 feet (60.96 cm.) three times each week; the lamp was run fifteen minutes before the animals were placed under it, and food and water were removed from the cage during irradiation.

dosage sufficient, as will be noted later, to supply adequate vitamin D.[3] All vitamin D preparations were added to the dry diets freshly twice each week. Diets and water were fed ad libitum.

2. Since this paper was submitted for publication it has come to our attention that viosterol 250 D has one hundred instead of two hundred and fifty times as much vitamin D as the cod liver oil used in these experiments (Bills, C. E.: Physiol. Rev. **15**:1, 1935; personal communication to the authors). On this basis the vitamin D content of diet 66 is equivalent to 0.8 instead of 2 per cent of cod liver oil, and that of diet 67 is equivalent to 2 instead of 5 per cent of cod liver oil.

3. The Hanovia Manufacturing Company furnished the lamp used in these experiments.

As in previous experiments, rats were bred on our modified McCollum stock ration and distributed among the experimental groups twenty-two days after birth. Litter-mates were distributed separately in the two series. Each diet group contained from 10 to 13 animals. The period of experimental feeding ranged from forty-five to one hundred and eighty days; at intervals during this period animals were removed from the experimental groups in lots of litter-mates, bled by cardiac puncture for analyses of calcium and phosphorus and killed with chloroform. One side of the mandible of each animal was used for the preparation of decalcified (celloidin) sections stained with hematoxylin and eosin. Generally the side that showed the fewer macroscopic lesions was selected for this purpose. The analytic methods employed were those described in the previous report in this series,[1c] in which the method used for identification and rating of lesions of caries was also described in full. Lesions are rated according to size and degree of penetration from 1 to 10. The "index" value, as applied in this and the previous report, is obtained by dividing the total caries score for the group by the number of animals in the group. This value provides a satisfactory measure of the incidence of caries in the experimental groups, as indicated by its relatively close reproducibility. Lesions identified as resulting from fracture of a cusp, including those referred to by other workers as macroscopic caries, were recorded in these studies as such, but are not included in the tables, which deal only with fissure caries.

RESULTS

Table 2 presents data for the individual groups on the incidence of caries, calcification of teeth and bone and blood calcium and phosphorus. The values for caries are given in terms of both the percentage of animals affected in the group and indexes. The two sets of values are not well correlated, since only the index values reflect the number and size of the lesions as well as their distribution by animals. Data on calcification, as determined by examination of the sections, are given here roughly only. All the pathologic changes observed have been previously described by ourselves and other investigators. It will be noted that the occurrence and kind of defects of calcification were those to be expected from the character of the respective diets. The low calcium diets resulted in uniformly defective dentin, whereas the high calcium diets produced local defects in calcification, generally over the pulpal floor and in the roots of the molars. Diets 66 to 69, which contained vitamin-oil preparations as the only additions to a basal diet very low in minerals, produced molar dentin that was slightly defective locally and alveolar bone normal in quality but apparently reduced in amount. The same was true of the group that received ultraviolet irradiation (72). The blood values likewise parallel closely the histologic observations and are in the expected range for the several diets. It is interesting that the blood values for the two diets containing rice passed through a 100-mesh sieve (58 and 61) both indicate somewhat more severe deficiency than the respective diets with rice passed through a 10-mesh sieve. Growth was recorded as a check on the condition of the animals during the course of experimental feeding, but the method employed

of removing animals from each group at intervals during the progress of the experiment produces average curves that are not strictly comparable. Hence curves for growth are not presented.

In order to assess the significance of the reduced caries indexes of the experimental groups, as compared with the control groups, the index values have been treated statistically.[4] As a basis for this treatment all groups fed the control diet (with rice passed through a 10-mesh sieve, 92 per cent; dextrin, 8 per cent, plus from 3 to 5 Gm. of spinach per rat per day and water ad libitum) since these experiments

TABLE 2.—*Data on Incidence of Caries, Calcification and Blood Calcium and Phosphorus Values in Diets 55 to 72* *

Diet Group	Number of Animals	Additions to Basal Diet	Experimental Period, Days	Calcification		Caries		Blood Calcium, Mg. per 100 Cc.	Blood Phosphorus, Mg. per 100 Cc.
				Teeth	Bone	Percentage of Animals	Index		
55	12	High calcium, high phosphorus	45-177	N	N	67	2.9±1.0	10.90±0.37	6.04±0.16
56	12	Diet 55 + 2 per cent CLO.....	45-177	N	N	50	2.3±1.0	10.75±0.13	7.16±0.31
57	12	None.....	45-120	VD	VD	75	6.3±2.4	5.52±0.32	8.30±0.78
58	10	None (100 mesh)	45-120	VD	VD	0	0	5.13±0.35	8.75±0.40
60	10	High calcium....	44-108	LD	VD	90	4.5±1.6	9.55±0.54	3.92±0.29
61	10	High calcium (100 mesh).....	44-115	LD	VD	0	0	11.15±0.33	3.68±0.31
63	11	Low calcium.....	45-170	D	D	45	3.2±1.6	6.47±0.16	6.90±0.25
64	11	7 per cent protein	45-105	VD	VD	82	5.4±1.3	5.47±0.20	7.56±0.25
65	12	None.....	55-181	VD	VD	100	7.8±1.5	5.62±0.30	8.08±0.36
66	12	Vios. in CO, 2 per cent.....	55-181	LSD	NR	33	2.1±1.1	10.16±0.41	6.23±0.16
67	13	Vios. in CO, 5 per cent.....	47-181	LSD	NR	31	0.9±0.5	10.38±0.30	7.67±0.22
68	10	2 per cent CLO...	47-181	LSD	NR	50	2.3±1.3	10.75±0.12	7.58±0.34
69	13	5 per cent CLO...	47-181	LSD	NR	46	3.1±1.5	10.30±0.23	7.43±0.50
70	11	2 per cent CO....	49-181	VD	VD	82	3.0±0.9	6.22±0.24	8.26±0.67
71	12	5 per cent CO....	47-181	VD	VD	50	2.8±1.3	5.49±0.17	7.13±0.52
72	12	Irradiated.....	55-181	LSD	NR	58	4.5±1.4	10.61±0.20	7.26±0.28

* In this and other tables the following abbreviations are used: CLO, cod liver oil; CO, corn oil; Vios., viosterol; N, normal; D, defective; VD, very defective; LD, locally defective; LSD, locally slightly defective; NR, normal in quality but reduced in amount. The ± values are standard deviations.

were started in 1931, with one exception, were pooled to form a group of 94 animals. The exception was the group on diet 24 (1934), which was omitted as clearly aberrant. This control group was then pooled individually with each experimental group to be compared to obtain a mean index (m_o) and its standard deviation. The standard deviation of the difference between the index of the experimental group and

4. Prof. Earle B. Phelps of the Department of Public Health of the College of Physicians and Surgeons collaborated in formulating the statistical method used. The method is based on the assumption of homogeneity of the different groups pooled to obtain a mean index, parts of the pooled group being then compared with the whole to test this assumption.

the mean index was derived as the square root of the sum of the squared standard deviations of the two means. The ratios of these differences to their standard deviations were interpreted from the table given by Pearl.[5] The following equations were employed:

Standard deviation of an index $(\sigma_m) = \sqrt{\frac{\Sigma d^2}{N(N-1)}}$

Standard deviation of a difference $(\sigma_D) = \sqrt{\sigma_o^2 + \sigma_m^2}$

Mean of a series of (two or more) component means $(m_o) = \frac{\Sigma mf}{N}$

Standard deviation of m_o $(\sigma_o) = \sqrt{\frac{\Sigma(\Sigma d^2 + [f(m_1 - m_2)^2])}{N(N-1)}}$

TABLE 3.—*Significance of Reductions in Caries Produced on Addition of Calcifying Foods, Corn Oil or Protein to the Basal Deficient 10-Mesh Rice-Dextrin-Spinach Diet*

Diet Group	Additions to Basal Diet	Number of Animals	Calcification, Blood Calcium and Phosphorus	Caries Index, m	m_o	$m_o - m = D$	$\frac{D}{\sigma_D}$	Odds Against Random Occurrence of Difference
Pooled control	None.....	94	D*	7.6±0.6				
55	High calcium, high phosphorus.....	12	N	2.9±1.0	7.1±0.6*	4.2±1.2	3.5	2,149 to 1
56	Diet 55 + 2 per cent CLO.....	12	N	2.3±1.0	7.0±0.6	4.7±1.2	3.9	10,390 to 1
60	High calcium.....	10	D	4.5±1.3	7.3±0.5	2.8±1.4	2.0	21 to 1
63	Low calcium.....	11	D	3.2±1.6	7.1±0.5	3.9±1.7	2.3	46 to 1
64	7 per cent protein..	11	D	5.4±1.3	7.4±0.5	2.0±1.4	1.4	5 to 1
66	Vios. in CO, 2 per cent.....	12	N	2.1±1.1	7.0±0.5	4.9±1.2	4.1	30,000 to 1
67	Vios. in CO, 5 per cent.....	13	N	0.9±0.5	6.8±0.5	5.9±0.7	8.4	∞ to 1
68	2 per cent CLO.....	10	N	2.3±1.3	7.1±0.5	4.8±1.4	3.4	1,483 to 1
69	5 per cent CLO.....	13	N	3.1±1.5	7.1±0.5	4.0±1.6	2.5	80 to 1
70	2 per cent CO.....	11	D	3.0±0.9	7.1±0.5	4.1±1.1	3.7	4,637 to 1
71	5 per cent CO.....	12	D	2.8±1.3	7.1±0.5	4.3±1.4	3.1	516 to 1
72	None; irradiated...	12	N	4.5±1.5	7.2±0.5	2.7±1.6	1.7	10 to 1
25, 66, 67, 68, 69	Vios. in CO or CLO	55	N	1.9±0.5	5.5±0.5	3.6±0.7	5.1	4,000,000 to 1
27, 28, 37, 38, 56	Normal calcium and phosphorus + 2 or 5 per cent CLO..	38	N	1.6±0.5	5.9±0.5	4.3±0.7	6.1	700,000,000 to 1
70, 71	2 or 5 per cent CO..	23	D	2.9±0.8	6.7±0.5	3.8±0.9	4.2	50,000 to 1

* See footnote, table 2.

In these equations σ indicates the standard deviation; D, the difference (between two means); d, the deviation (of an individual value from the mean); N, the number of animals in the whole group considered; f, the number of animals in a component group, and m, the mean (or index).

The results of this analysis are given in table 3, in which data on calcification and dietary composition are repeated to facilitate comparisons. The reduction of caries obtained with diets 55 and 56, containing

5. Pearl, R.: Introduction to Medical Biometry and Statistics, Philadelphia, W. B. Saunders Company, 1930.

calcium and phosphorus in high levels and a normal ratio, without cod liver oil and with 2 per cent of cod liver oil, respectively, are clearly significant; while those obtained with diets 60, 63 and 64 are not. The results obtained with the diets containing rice passed through a 100-mesh sieve (58 and 61) are not included in the table. Outright prevention of caries was obtained with both these diets. The group of diets in which vitamin D in oil was present either as viosterol or in cod liver oil produced reductions the odds against the insignificance of which range from 80 to 1 to a value approaching infinity to 1, and it is interesting that the extremes of this range both fall in the groups that received the higher percentage of the vitamin-oil preparations. The results obtained with diets 70 and 71, which were set up as controls for the inert oil in diets 66 to 69, are surprising, since a clearly significant reduction of caries was obtained with each.

Since the standard deviation decreases as the number of animals in the group is increased, small differences become more significant as the size of the group is increased. This fact is apparent in the comparisons for the pooled groups given in the lower part of table 3. The mean difference between the control group and the groups that received vitamin D-oil preparations as the sole additions to the basal diet (diets 66 to 69, plus diet 25—containing 2 per cent of cod liver oil—from an earlier series) is clearly significant. Similarly, when group 56 is pooled with previous groups that received diets containing normal calcium and phosphorus plus 2 or 5 per cent of cod liver oil (27, 28, 37 and 38) and when the two groups fed the corn oil diet (70 and 71) are pooled, the mean differences are clearly significant.

It is of further interest to determine whether the groups that received calcifying foods, corn oil or ultraviolet irradiation differ significantly with respect to each other. To determine this, the entire series of 147 animals, including groups on diets 25, 26, 27, 28, 37 and 38, in addition to the diets described here which yielded significantly reduced indexes of caries as compared with the control, were pooled to derive a mean index and standard deviation. When the indexes of the component groups are compared with the index of the complete series by the method previously used, it becomes clear that little weight can be attached to differences between these groups. This analysis is shown in table 4. It is interesting that the reduction of caries obtained with diets 70 and 71, although less in degree than the mean reduction obtained with the adequately calcifying diets, nevertheless falls within this observed variation. It is also noteworthy that in group 72 (irradiated animals) the result does not differ significantly from this complete series or from the control group (table 3).

To determine whether the blood values for calcium and phosphorus are related to the incidence or degree of caries a similar method of

analysis was used. In this instance, however, it is obvious that the abnormal values of animals that received deficient diets could not be used in the analysis, for although they would appear to be directly related to the caries indexes the fact that similar abnormal values were obtained with the diets containing rice passed through a 100-mesh sieve in the complete absence of caries clearly indicates that such abnormal values do not necessarily imply the occurrence of caries. It seemed valid rather to determine whether the variation in the blood values for calcium and phosphorus among the groups in which normal averages were obtained are related significantly to the degree of caries shown by those animals individually. Thus, 86 animals for which analytic data are available were treated as a unit as before, and mean values and standard deviations derived for calcium and phosphorus. This group

TABLE 4.—*Comparative Influence of Calcium, Phosphorus, Vitamin D and Corn Oil on Experimental Dental Caries in Rats; Significance of Differences Between Experimental Groups*

Diet Group	Additions to Basal Diet	Number of Animals	Calcification, Average Calcium to Phosphorus Ratio	Calcification, Blood Calcium and Phosphorus	Caries Index	$\frac{D}{\sigma_D}$	Odds Against Random Occurrence of Difference
Complete series	.....	147	....	..	2.5±0.3		
25, 66, 67, 68, 69	Vios. in CO or CLO	55	0.12	N*	1.9±0.5	0.9	2 to 1
27,28, 37, 38, 56	Normal calcium and phosphorus + 2 or 5 per cent CLO.....	38	1.31	N	1.6±0.5	1.3	4 to 1
70, 71	2 or 5 per cent CO...	23	0.12	D	2.9±0.8	0.4	Less than 1 to 1
72	None; irradiated....	12	0.12	N	4.5±1.4	1.1	3 to 1

* See footnote, table 2.

was then subdivided, without regard to experimental diets, into those with no caries and those with caries equivalent to a score from 1 to 3, 4 to 6 and 7 or more, respectively. When these groups are compared with the complete series, with the exception of calcium in the group with the greatest incidence of caries, the differences are less than their standard deviations, and it is clear that none is significant.

COMMENT

The result obtained with diet 58 confirms our previous finding that caries is prevented outright in the low calcium diet when the rice is ground to pass through a 100-mesh sieve. The absence of caries with diet 61 indicates that the same is true in the case of a high calcium diet. It is to be noted that each of these diets was not less severe in nutritional character than the corresponding diet containing rice passed through a 10-mesh sieve. It is also noteworthy that the high calcium

diet containing rice passed through a 10-mesh sieve (60) produced less caries than the corresponding low calcium diet, and although the difference is not significant it is clear that a relative deficiency of phosphorus is not more productive of caries than a deficiency of calcium. Addition of calcium and phosphorus to the basal diet, such as to produce a slight improvement in ratio but leaving the diet (63) relatively low in calcium, also resulted in a decrease of caries of doubtful significance. Addition to the basal diet of 7 per cent of protein in the form of wheat gluten (diet 64) did not produce a significant change in the incidence of caries, suggesting that the protein deficiency in the basal diet is not a factor in the production of the lesions.

The findings as a whole, particularly with the corn oil and more adequately calcifying diets, may be considered as they bear on three aspects of the problem: the comparative influence of the calcifying

TABLE 5.—*Relationship of Blood Calcium and Phosphorus to Experimental Dental Caries in Rats; Significance of Differences Between Experimental Groups Showing Generally Normal Values*

Diet Group	Number of Animals	Calcium			Phosphorus		
		Mean	$\frac{D}{\sigma_D}$	Odds Against Random Occurrence of Difference	Mean	$\frac{D}{\sigma_D}$	Odds Against Random Occurrence of Difference
Complete series.....	86	10.56±0.08	...	.....	7.22±0.12	...	.....
Caries score = 0.....	50	10.49±0.09	0.6	1 to 1	7.25±0.17	0.2	Less than 1 to 1
Caries score = 1-3.....	12	10.37±0.21	0.9	2 to 1	7.22±0.31	0.0	0
Caries score = 4-6.....	10	10.43±0.18	0.7	1 to 1	7.24±0.29	0.1	Less than 1 to 1
Caries score = 7 or more	14	11.02±0.28	1.6	8 to 1	7.00±0.31	0.7	1 to 1

elements, vitamin D and oil; the influence of varying amounts of these substances, and the mechanism whereby they act to reduce the incidence of caries.

The finding that corn oil, supplementing a diet very deficient in minerals and without vitamin D, produced a reduction of caries statistically equivalent to that produced by adequately calcifying diets must lead one to doubt the importance of vitamin D in this connection. Likewise, the effect on caries observed on ultraviolet irradiation, although inconclusive, helps to sustain the implication that the fat content of the vitamin D preparations rather than their vitamin content is the more potent protective agent against caries. The fact that calcium and phosphorus in high levels and a normal ratio in the absence of vitamin D or oil (diet 55) produced a significant reduction in caries, on the other hand, prevents exclusion of the calcifying elements as participants in this protective effect.

In considering the bearing of our data on the quantitative aspects of the caries-reduction phenomenon, it may be important to emphasize

that all the evidence obtained points to the coarse cereal particles as the essential caries-producing agents. When the basal coarse rice diet is supplemented with various foods, whether or not they improve its nutritional quality, the result is not more than partial reduction of caries; outright prevention regularly occurs only when the coarse cereal is ground to a flour. It is clear from these experiments that this relative protective effect may be contributed either by vitamin D-oil preparations, by calcium and phosphorus in normal ratio, by corn oil or by calcium and phosphorus in normal ratio with vitamin D in oil. The last-named combination produced the greatest average reduction in caries (table 4), but the statistical analysis indicates that none of these agencies can be regarded as superior to the others. The results obtained with diets 66 to 69, particularly, suggest that 5 per cent of the vitamin D-oil preparations is not necessarily more effective than 2 per cent, and the result in the case of the single diet 56 further suggests that even in the presence of what may be regarded as optimal dietary conditions —calcium and phosphorus in high levels and a normal ratio, with 2 per cent of cod liver oil—the protective effect cannot be depended on to exceed that produced by 2 per cent of cod liver oil or its viosterol or fat equivalent alone.

We have pointed out before that although adequately calcifying diets tend, in general, to produce less caries than comparable deficient diets, the difference in caries cannot be directly related to corresponding differences in the picture of calcification. The data recorded here confirm this point of view and make it clear as well that variations in blood calcium and phosphorus, within a generally normal range, do not reflect variations in the incidence of caries in individual animals. The mechanism underlying these facts is not clear. If the results of a given deficient caries-producing diet are compared with those obtained with a similar diet in which the deficiencies are corrected, the caries values in the two groups will appear to be related to these differences, to the degree of calcification determined histologically and to the blood values of calcium and phosphorus. Yet the results in this study of the experiments with rice passed through the 100-mesh sieve, incorporating both types of calcifying defect, each of which was reflected characteristically in the bone, teeth and blood, show that such deficiency may be present in the entire absence of caries. And the fact that the addition of corn oil to the basal diet, although it effected no abatement of its calcifying deficiency, produced a reduction of caries equivalent to that obtained with adequately calcifying diets, provides additional evidence pointing in the same direction. Finally, the circumstances that the degree of protective effect exerted by the calcifying foods and corn oil was not related to the amounts of these substances in the diet and particularly that a diet with fully adequate calcifying properties (56)

was not more effective in reducing caries than diets that produced somewhat imperfect calcification (66 to 69) suggest that these agencies operate by way of some mechanism other than that of calcification. We are hardly justified in concluding that these protective effects are exerted directly on the environment of the teeth rather than by way of systemic or metabolic channels, yet—excepting the influence of ultraviolet irradiation, which these results leave doubtful—the evidence does not invalidate the view that such may be the case.

SUMMARY AND CONCLUSIONS

This report deals with the results obtained with 286 animals, including 94 that received the (control) deficient caries-producing diet of rice passed through a 10-mesh sieve, dextrin and spinach and 192 that received various modifications of this diet intended to test the influence on the production of caries of calcifying agents and corn oil and the relative importance of the size of particles of rice and the two kinds of dietary calcifying defect. Of these animals 183 are included in the experimental and control groups described here in full; the remainder have been described elsewhere and were used here for comparative purposes. All the results are analyzed statistically. The results appear to warrant the following conclusions:

Caries is produced with the coarse rice basal diet either of the high calcium deficient type or of the low calcium type. The high calcium diet is not more effective in producing caries than the low calcium diet.

With both types of diet the incidence of caries becomes zero when the rice is ground to pass through a 100-mesh sieve.

Addition to the basal diet of cod liver oil or viosterol in corn oil as 2 per cent of the diet produced a definite reduction in caries, but did not prevent caries outright. The protective effect was not significantly augmented by increasing the dosage to 5 per cent of cod liver oil or viosterol in corn oil or by the further addition of calcium and phosphorus to a normal ratio, with levels normal or definitely high.

Irradiation of animals on the basal diet with ultraviolet rays in a dosage sufficient to produce improvement in calcification as great as that produced by 5 per cent of cod liver oil was less effective in reducing caries than feeding vitamin D, but this result is statistically inconclusive.

Addition to the basal diet of 2 per cent or 5 per cent of corn oil did not remedy the dietary deficiencies but nevertheless produced a significant reduction in caries statistically not distinguishable from that produced by feeding vitamin D in oil. Thus at least part of the protective effect of vitamin D-oil preparations appears to be contributed by the vitamin-free oil.

Within the groups that received diets with adequate or nearly adequate calcifying properties, individual variation in the incidence of caries was found to be unrelated to the blood levels of calcium or phosphorus. Likewise, the degree of protective effect against caries of the diets that were supplemented with calcifying agents was not related to the degree of their calcifying action. From the evidence as a whole it appears that the protective effect against caries exerted by the calcifying foods may be independent of the mechanism of calcification and possibly of metabolic influences generally.

SPONTANEOUS ARTERIOSCLEROSIS IN RATS

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In view of the complete lack of information in regard to the occurrence and frequency of spontaneous arteriosclerosis in rats (Duff[1]), the following report may be of interest.

In the course of routine histologic examinations of the organs of seventy-five rats used for experimental purposes, arteriosclerotic lesions were seen in the branches of the pulmonary artery in twelve. On account of the relatively small size of the lesions and the absence of serial sections it is probable that the incidence of arteriosclerosis of the pulmonary artery is higher than is apparent from these figures. In none of the other organs (brain, heart, ascending part of the aorta, liver, spleen, pancreas, adrenal gland, kidney, testis and bladder) were such changes observed. The rats were adult and all had been obtained from one dealer, but no information is available as to whether they belonged to one stock. As their death was the result of poisoning with a chemical that killed the animals within a few hours or days it is unlikely that an exogenous chemical factor was the cause of or contributed to the production of these lesions. They were found in the large and medium-sized branches of the pulmonary artery and were most frequently located at or near the spur of bifurcations of this vessel. While in the majority of the cases multiple plaques were shown in the walls of one or several vessels, occasionally only a single calcified focus was seen.

The greater number of the lesions consisted of cone-shaped calcified foci covered by endothelium and projecting into the lumen, being located in the subendothelial tissue and extending rather frequently into the media, which showed evidence of local hyaline degeneration. In some instances the calcified foci were indistinctly outlined streaklike formations involving only the media. Small hyalinized areas, sometimes containing minute central calcifications, were occasionally observed involving the media. The intima covering these areas was often thickened and the seat of cellular proliferation. It was noticed that there existed a more or less marked hypertrophy of the media in many of the smaller branches. Complete obliteration of the lumen of a medium-sized vessel with a large calcium deposit in the partially hyalinized and sclerotic wall was found in one instance. As these observations were made on paraffin sections exclusively no statements can be made as to the presence of deposition of lipoids in the vascular walls.

SUMMARY

Arteriosclerotic lesions in the walls of the branches of the pulmonary artery of the white rat are described.

From the Haskell Laboratory of Industrial Toxicology.

1. Duff, G. Lyman: Arch. Path. **20**:81, 1935.

MOUSE LEUKEMIA

IX. THE RÔLE OF HEREDITY IN SPONTANEOUS CASES

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The general question of the relative potency of heredity and environment must be replaced by a specific question for each specific case. For all degrees of relationship between these categories of controlling influences are now known to exist. Thus the distinction between so-called hereditary and nonhereditary traits is not clearcut nor does it represent a fundamental difference. If the hereditary variables are relatively strong, clearcut analyses in terms of specific genes are possible; when nonhereditary variables are strong, such analyses are rarely possible. Between these limits lie all degrees of genetic potency and hence all degrees of reliability of genetic interpretation.

In the case of leukemia the evidence from human records seems to indicate that heredity plays a very minor rôle (Ardashnikov,[2] Petri [3]). In mice, the leukemia of which shows the closest possible resemblance to the human disease and its variations, heredity can be shown to have an unquestionable influence.

The only published discussion on heredity in relation to the spontaneous leukemia of mice (Slye [4]) proposes a clearcut genetic interpretation on the basis of "perfect" and "nearly perfect" mendelian ratios. The results herein presented confirm the existence of a hereditary predisposition to leukemia, but also show that in this case nonchromosomal variables play such a conspicuous rôle that genetic ratios can be determined only by breeding tests of the individual mice in a segregating generation, not by their own somatic conditions, and, further, that even within the limits of the highly controlled experiments described

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From the Department of Genetics, Carnegie Institution of Washington, and the Department of Pathology, College of Physicians and Surgeons, Columbia University.

1. Footnote deleted by the author.
2. Ardashnikov, S. N.: Proc. M. Gorky Med. & Biol. Research Inst. **3**:169, 1934.
3. Petri, S.: Acta path. et microbiol. Scandinav. **10**:330, 1933.
4. Slye, M.: Am. J. Cancer **15**:1361, 1931.

nonchromosomal variables cannot be eliminated as constant, for their effectiveness varies according to the genetic constitution of the mice.

LEUKEMIC STRAIN C58

In earlier papers of the present series (Richter and MacDowell;[5a] MacDowell and Richter[6]) reference has been made to the frequent occurrence of spontaneous leukemia in an inbred strain of mice designated as C58. This strain has provided most of the material for these studies. The ancestors of strain C58 were brought to the department of genetics in 1920 by Dr. C. C. Little. They were received from Mrs. Gray to whom were turned over the stocks of Mrs. Abby Lathrop. These stocks were derived from those studied in cooperation with Dr. Leo Loeb (Lathrop and Loeb[7]). The animals constituted a genetically heterogeneous group; they were of various colors—black, brown, yellow, brown agouti; subsequent inbreeding resulted in the isolation of several strains with characteristic traits, such as defects in development, a peculiar behavior pattern and the predisposition to leukemia. Since 1920 the matings in this strain have been between brother and sister; in the early generations blacks and browns occurred, but the direct ancestors of the recent generations have been homozygous black since the very earliest generations of inbreeding. Before the eighteenth generation several animals with greatly enlarged spleens were casually noted. Their occurrence in this strain was recognized as characteristic, but no particular attention was paid to them, and the records tell nothing about the incidence since most of the animals were killed as soon as breeding became reduced. This was usually early, for another characteristic of this strain was early maturity followed by unusually heavy deposition of fat and a short breeding period. A few animals with large spleens and livers were preserved, and from sections their condition was subsequently diagnosed by Dr. Alwin M. Pappenheimer as lymphatic leukemia.

In 1928, when the cooperative project on leukemia was undertaken by the two departments, all the animals of this strain not required for special experiments were permitted to die naturally until a group of 637 animals that lived at least six months was collected. Since the animals used in the special experiments were taken entirely at random, this was a purely random sample of the strain. These mice were born in generations 18 to 23. A second random sample consisted of 70 mice from generations 26 to 28, born in March and April 1931.

5. Richter, M. N., and MacDowell, E. C.: (*a*) Proc. Soc. Exper. Biol. & Med. **26**:362, 1929; (*b*) J. Exper. Med. **53**:823, 1930.

6. MacDowell, E. C., and Richter, M. N.: Biol. Zentralbl. **52**:266, 1932.

7. Lathrop, A. E. C., and Loeb, L.: J. Exper. Med. **28**:475, 1918.

DIAGNOSES

All these mice died and their disorders were diagnosed by one of us (M. N. R.) with the aid of the gross autopsy records and microscopic sections of the principal organs and tissues fixed in Zenker's fluid and stained with hematoxylin and eosin.

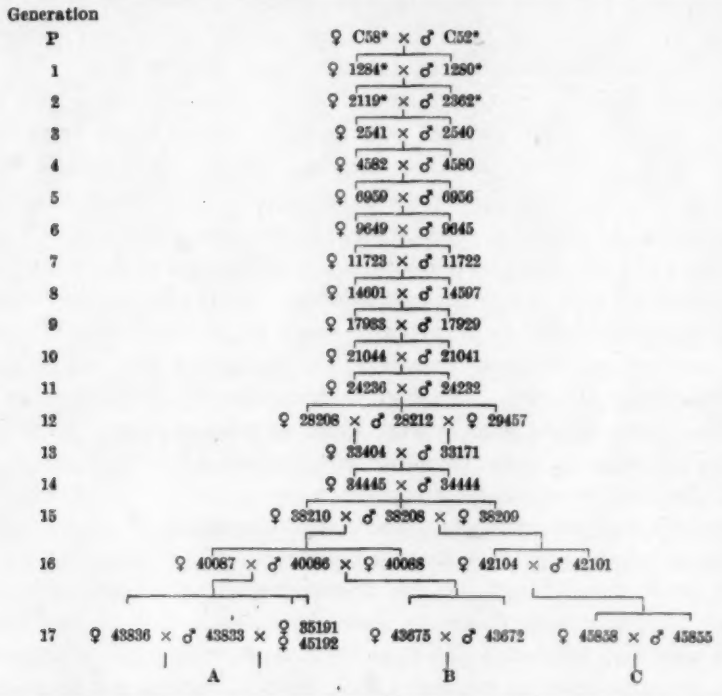
The occurrence in this highly homogeneous strain of mice of various conditions involving malignant leukocytic infiltrations, recognized by different descriptive names, justifies the use of one term to cover the group. In the absence of any term entirely satisfactory for this purpose we have used the term "leukemia" as a matter of convenience, believing that definitive terminology can be established only at the completion of experimental analysis. Thus in the tables and pedigrees leukemia and related conditions are all classed as leukemia.

Serious difficulty was encountered in the diagnosis of certain conditions owing to rapid changes post mortem, and to death from other causes with leukemia in some incipient stage. Occasionally other puzzling histologic conditions were encountered that defied diagnosis. Thus a class of doubtful cases arose. In the first sample of 637 mice 99 cases (15.5 per cent) were doubtful; in 63 per cent of these the doubt was manifestly due to changes post mortem. Thirty-five of the doubtful cases were considered to be probably cases of leukemia, and 20, probably cases of nonleukemic disorders; no probability was indicated for the remaining 44 cases. In order to minimize the doubtful cases the animals in the second sample were killed as soon as clear clinical indications of leukemia were found or on approach of a moribund condition. In this way definite diagnoses were obtained for all except 2 mice which died unexpectedly. The virtual elimination of doubtful cases in this sample may indicate that slight changes post mortem can lead to doubt or that doubt may be due to terminal complications of various sorts. Breeding tests (table 4) showed that mice with doubtful diagnoses were not genetically different from those with positive diagnoses. Thus doubtful cases as a whole appear to represent an accidental class without significance for this study. So far as this is true they form a random sample, and their elimination from the calculations of the incidence of leukemia appears to be the least unsatisfactory method of dealing with them. When so calculated the incidence of leukemia in the two samples from strain C58 becomes 90.1 per cent and 85.3 per cent of the definite diagnoses.

In the great preponderance of the cases the leukemia was of the lymphatic type. Out of a grand total of 543 cases in which leukemia was positively diagnosed, in 450 the condition was lymphatic (in 19 of these, lymphosarcomatous); in 6, myeloid, and in 87, doubtful as to cell type. In 55 of the doubtful cases the condition was called "prob-

ably lymphatic" and in 3 "probably myeloid." The doubt as to cell type was referable to changes post mortem in 71 cases. The cases of myeloid leukemia were not concentrated in any part of the inbred pedigree, and they appeared occasionally in both hybrid generations of the cross reported. Since nothing can be said at present as to the etiologic relations of lymphatic and myeloid leukemia the distinction is not made in the numerical statement of results.

TABLE 1.—*The Ancestors of the Mice Studied, Showing the Relationship of the Sections Indicated as A, B and C*



* Animals in the first three generations were recorded in the pedigree book of Dr. C. C. Little while he was in the department of genetics.

GENETIC HOMOGENEITY OF STRAIN C58

Inbreeding leads to genetic uniformity. Fourteen generations of brother and sister matings theoretically insures a high degree of homozygosity. Thus in strain C58, 14 generations of such inbreeding (table 1) resulted in a common genetic constitution that on sufficient observation proved to predispose to leukemia. But even so leukemia was not manifest in all mice of the strain; 10 per cent of the definite diagnoses were negative for leukemia. The inference to be drawn is that differences represented in the negative and positive cases are not genetic

differences. This can be checked in two ways: If there are no genetic differences between leukemic and nonleukemic mice in this strain the nonleukemic mice should be scattered at random through the different families and branches of the pedigree and the offspring of the nonleukemic parents should give the same incidence of leukemia as the offspring of the leukemic parents.

TABLE 2A.—*The Leukemic, Nonleukemic and Doubtful Offspring in the First Sample of Six Hundred and Thirty-Seven Mice from Strain C58, Generations 18 to 22, Classified by Fathers*

Generation		Fathers*	Offspring†		
			+	−	?
16		38208			
17		40086			
18	A	43833	8	0	0
19		48387	3	1	0
19		46864	19	3	2
20		50418	43	7	9
21		52437	17	1	2
22		53449	10	2	3
23		55252	12	2	0
22		53947	8	1	1
21		52649	28	2	10
22		53641	8	0	2
22		54296	7	0	1
22		53567	3	1	0
21		53376	5	1	0
22		54396	2	1	5
21		52708	29	5	5
22		54101	5	0	1
22		53705	5	0	1
20		52489	38	1	2
21		53320	8	1	1
			248	29	45
18	B	43672	6	1	2
19		46617	25	5	8
20		49352	28	1	9
21		52628	3	0	1
21		53245	12	1	2
20		52447	2	0	0
20		49150	26	1	3
21		52535	11	0	3
21		52769	9	2	1
20		53508	0	1	1
19		49717	34	2	6
20		52496	40	4	5
21		54063	2	0	1
			198	18	42
17		42101			
18	C	45855	4	2	0
19		49917	34	3	10
20		53230	1	1	2
			39	6	12
Total			485	53	99

* A son is below his father and one step to the right.
† The plus sign means leukemic; the minus sign, nonleukemic; the question mark, doubtful as to leukemia.

Evidence on these points is given in tables 2 to 4. Table 2 summarizes the data from the first group of 637 mice according to individual fathers and table 3 according to generations. It is clear that the nonleukemic as well as the doubtful leukemic offspring were distributed through all the pedigree; this is direct evidence of genetic homogeneity.

Table 4 classifies all the matings according to the diagnoses of conditions in the parents. While the number of nonleukemic parents is necessarily small, it is none the less certain that matings involving 1 or 2 nonleukemic parents yielded fully as large proportions of leukemic offspring as matings between 2 leukemic parents. Thus by direct breeding test nonleukemic animals in strain C58 were shown to be genetically the same as the mice that died of leukemia. From this it follows that heredity was strong enough to lead to leukemia in most of the mice of strain C58, but 10 per cent of them met conditions that prevented the manifestation

TABLE 2B.—*Leukemic, Nonleukemic and Doubtful Offspring in the Second Sample of Seventy Mice from Strain C58, Generations 26 to 28, Classified by Fathers, Giving the Connection with the Earlier Sample*

Generation	Fathers	Offspring		
		+	−	?
21	52649			
22	54296			
23	56619			
24	61398			
25	66388			
26	70130			
27	77071	4	0	0
25	64450			
26	67260			
27	70324	4	1	0
21	52708			
22	56301			
23	60364			
24	61703			
25	64324			
26	68890			
27	72085	7	1	1
28	78503	4	0	1
26	72948	12	4	0
27	69495			
28	77942	13	0	0
25	64378			
26	67143			
27	72834	5	0	0
26	68767			
27	77407	5	4	0
27	77412	4	0	0
Totals				
Generation 26		12	4	0
Generation 27		29	6	1
Generation 28		17	0	1

of the hereditary tendency. One could say that in strain C58 heredity was 9 times as potent as nongenetic variables in controlling the incidence of leukemia. It might be supposed that death from some independent cause before leukemia could manifest itself accounts for the negative cases. This cannot have played any primary part since the animals not showing leukemia tended to live longer than those with the disease (average age at death of 63 nonleukemic animals, 495.5 days; average age of 543 leukemic mice, 396.4 days); 55 leukemic mice died earlier than the youngest nonleukemic one, and the oldest 3 mice were without sign of this condition; 47 per cent of the leukemic mice died

before 360 days, as compared with only 22 per cent of the mice free from this disease.

All the mice of strain C58 inherited a tendency toward malignant change in the leukocytes. But even among those with leukemia there was indication of the action of nongenetic influences since different mice showed considerable range in the malignant manifestation. Transmission experiments have proved the existence of lines of malignant lymphocytes with many different traits (Richter and MacDowell;[5b] Victor and

TABLE 3.—*Spontaneous Leukemia in Strain C58: A Summary of the Diagnoses According to Branch of Strain and According to Inbred Generation*

Ancestor	Diagnoses			Percentage of Definite Diagnoses Positive for Leukemia
	+	−	?	
♂ 43833	248	29	45	89.5
♂ 43672	198	18	42	91.7
♂ 45855	39	6	12	86.7
Generation				
18-19	133	17	28	88.7
20	168	16	31	91.3
21	124	13	26	90.5
22-23	60	7	14	89.6
18-23	485	53	99	90.1
26-28	58	10	2	85.3
Totals				
Males	240	30	54	88.9
Females	303	33	47	90.2
	543	63	101	89.6

TABLE 4.—*A Classification of the Offspring in Strain C58 According to the Presence or Absence of Leukemia in the Parents*

Parents	Offspring			Percentage of Definite Diagnoses Positive for Leukemia
	+	−	?	
+ × +	286	36	59	88.8
+ × ?	53	5	13	91.4
? × ?	11	0	1	100.0
+ × −	55	5	12	91.7
− × ?	4	0	0	100.0
− × −	10	0	3	100.0

Potter;[8] Furth, Seibold and Rathbone[9]); indications are strong that leukemic cells from different spontaneous cases are inherently different. Given uniformity of the strain, the source of these differences is apparently nongenetic.

Since the relation of leukemia to other neoplasms has been a matter of discussion, table 5 is presented, showing the distribution in the large sample from strain C58 of other neoplasms according to the diagnosis in regard to leukemia and according to the father. The neoplasms were

8. Victor, J., and Potter, J. S.: Proc. Soc. Exper. Biol. & Med. **30**:523, 1933.
9. Furth, J.; Seibold, H. R., and Rathbone, R. R.: Am. J. Cancer **14**:3, 1933.

distributed generally through the pedigree without respect to the occurrence of leukemia. Calling the offspring of one father by his sibs a family, one finds that of 35 families 17 disclosed no neoplasm besides leukemia while 18 each included 1 or 2 mice with the certain or questionable presence of some other neoplasm. Special attention should be called to 2 bone tumors as well as to the scarcity (2 cases) of mammary car-

TABLE 5.—*Occurrence of Other Neoplasms in Leukemic, Nonleukemic and Doubtful Offspring Classified According to Parentage*

Generation	Fathers	Neoplasms in Leukemic Offspring	Neoplasms in Nonleukemic Offspring	Neoplasms in Offspring in Which Leukemia Was Doubtful
16	38208			
17	40086			
18	43833			
19	48387			
19	46864	Carcinoma of lung	.....	Adenoma of liver
20	50418			
21	52437	.....	.....	Sarcoma
22	53449			
23	55252	.....	Sarcoma (?) of mesenteric node	
22	53947	.....	Neoplasm (?) of spleen	
21	52649	Carcinoma of breast		
22	53641			
22	54296			
22	53567	Carcinoma of lung		
21	53376			
22	54398			
21	52708			
22	54101			
22	53705			
20	52480	Carcinoma of lung	Carcinoma of kidney	
21	53320	Hemangioma and adenoma of liver		
18	43672	Adenoma of liver		
19	46617	Carcinoma of lung	.....	Adenoma of liver
20	49352	Sarcoma (?) of liver	.....	Epithelioma
21	52628			
21	53245			
20	52447	Carcinoma of breast		
20	49150	Adenoma of liver (?)	Adenoma of bile ducts	
21	52535	.....	.....	Chondro-osteosarcoma
21	52769	.....	Cylindroma of parotid gland	
20	53508	.....	Sarcoma (?) of node	
19	49717			
20	52496	Hemangioma of liver Osteoblastic sarcoma		
21	54063			
17	42101			
18	45855			
19	49917	Hemangioma of pancreas		
20	53250			
		13 (485) = 2.7%	6 (53) = 11.3%	5 (99) = 5.1%
			Total 24 (637) = 3.8%	

cinoma. The appearance of such sporadic cases of various neoplasms is commonly classified as nongenetic whereas it would be more correct to say that the constitution of mice of strain C58 is such that only under very unusual circumstances will such and such a neoplasm other than leukemia develop.

STRAIN STORRS-LITTLE

In order to prove the existence of hereditary influence it is necessary not only to demonstrate the occurrence of a trait in successive

generations of a given strain but to show that under the same external conditions strains with different constitutions give different results. Whereas several strains bred side by side with C58 have never shown a single case of leukemia, only one, StoLi (Storrs-Little), has been extensively studied. This is the strain that was not susceptible to inoculation with leukemic cells of line I (MacDowell and Richter [6]). The records of 306 autopsies between generations 14 and 29 of brother and sister mating have been studied. The outstanding neoplastic characteristic of this strain of pink-eyed dilute-brown mice was the incidence of mammary carcinoma. Among 87 autopsies on females in generations 14 to 19 were found 44 cases of mammary carcinoma; that is, 50.6 per cent of all females showed this neoplasm in comparison with 0.5 per cent of the females from strain C58. However, after generation 19 the incidence of mammary carcinoma was suddenly and inexplicably reduced to 4.7 per cent (in 169 females). There was no change in care, diet or room and there was no elimination of branches of the pedigree that could be connected with this change. For the whole series (256 females) the incidence of mammary carcinoma becomes 20.3 per cent. A few other tumors were scattered through the pedigree; most of these were carcinoma, but four (1.3 per cent of all autopsies) were diagnosed as lymphatic leukemia.

The isolated cases of leukemia in StoLi correspond in frequency to the isolated cases of mammary carcinoma in C58; they were nongenetic in the sense that they appeared only under very unusual conditions. But should other strains of mice be found the constitutions of which do not permit the appearance of either of these types of neoplasm under any conditions, they would provide a basis for measurement of the genetic influence in such so-called nongenetic cases.

Thus with a common environment there is established a difference between these two strains in the incidence of leukemia which is to be accredited to heredity and which is represented roughly by 90 per cent and 1 per cent. With these facts in hand a genetic analysis of this difference can be made by study of the segregation in generations following a cross between these two strains.

FIRST GENERATION HYBRIDS BETWEEN C58 AND STOLI

If a strain is genetically homogeneous all germ cells are genetically alike so that in the first generation of a cross between two homogeneous strains every fertilized egg must be genetically like every other one. Thus in the cross of strain C58 and strain StoLi all hybrids in the first generation are as genetically uniform as the parent strains. Table 6 shows that of 106 offspring with definite diagnoses from C58 fathers and StoLi mothers 42.5 per cent had leukemia, a reduction of virtually one half in the incidence of leukemia. This intermediacy in the incidence

of leukemia is not reflected in the character of the leukemia, for the cases cannot be distinguished from those in the pure bred strain, nor does this constitute an example of intermediate or blending inheritance, for in blending inheritance differences in genetic condition are indicated directly in somatic differences. Since the mice in this generation are genetically alike the presence or absence of leukemia must be decided by nonchromosomal variables. Thus in the first hybrid generation, as in the pure bred strain C58, the somatic manifestation of the individual mouse is not a direct measure of its genetic constitution. But in this generation the relative influence of nongenetic variables is more than 5 times as strong as in the inbred mice of strain C58. Closely parallel results from hybridization of strains of mice characterized by high and low cancer rates have been reported by Lathrop and Loeb.[7]

TABLE 6.—*First Generation (F_1) from Reciprocal Matings Between Eleven Males and Seventeen Females from Strain C58 and Six Males and Twenty-One Females of Strain StoLi*

	Number of Offspring from						Percentage of Definite Diagnoses Positive for Leukemia in Mice from		
	C 58 Mothers			StoLi Mothers					
Sex	+	—	?	+	—	?	C 58 Mothers	StoLi Mothers	Difference
Male	41	32	13	26	38	13	56.2	40.6	15.6
Female	45	21	14	19	23	21	68.2	45.2	23.0
Total							61.9	42.5	$19.4 \pm 4.3 = 4.5 \times$ P.E.

Among the nonchromosomal variables which modify the manifestation of leukemic heredity one important influence is transmitted through the mother alone. The results so far considered have not been subject to this influence since the discussion has been based on hybrids receiving the leukemic inheritance from their fathers. The corresponding figures for transmission through the mothers are 19.4 per cent higher, a difference of unquestionable statistical significance. A question of sex linkage naturally arises, for in a cross involving sex linkage reciprocal matings give different results in the first generation, but these differences are confined to the males alone. In this case the female offspring show as much or even more difference than the males. Thus sex linkage is eliminated.

Length of life (table 7) gives further evidence of difference between offspring from the reciprocal matings of this cross. The average difference, 138 days, is twelve times its probable error. Sex linkage is not involved since females show as great a difference as males (difference for males, 132.3 days; for females, 144.6 days).

Again evidence of nonchromosomal maternal transmission appears in the case of transmission of mammary carcinoma from strain StoLi. Excluding matings with StoLi after the nineteenth generation, when the incidence of mammary carcinoma became reduced, the difference (39.7 per cent more mammary carcinoma in daughters from StoLi mothers than in those from C58 mothers) is statistically significant (7.7 times the probable error). Thus in one form of the cross is found an excess of leukemia; in the other, an excess of carcinoma. This negative correlation between the two types of neoplastic disease indicates specific difference but similarity in the type of transmission. Some indication of such maternal influence on the cancer rate in hybrids was given by Lathrop and Loeb.[7] More recently several crosses reported by the Roscoe B. Jackson Memorial Laboratory (through C. C. Little [10]) involving wide differences in the incidence of mammary tumors show striking differences in the first generation from reciprocal matings.

TABLE 7.—*Average Length of Life in Days*

Parents	Offspring				
	Leukemic	Nonleukemic	Males*	Females*	Total
C 58.....	396.4 (543)†	495.4 (63)	447.5 (327)	384.1 (380)	413.4
StoLi.....	.....	563.9 (302)	548.2 (50)	586.5 (252)	563.9
StoLi/C 58.....	729.1	650.6	684.4	682.4	683.5
C 58/StoLi.....	554.3	503.7	552.1	537.8	545.2
Difference.....	174.8 ± 17.6	155.9 ± 20.0	132.3 ± 15.5	144.6 ± 17.1	138.3 ± 11.5
Difference/P.E.....	9.9	7.8	8.5	8.4	12.0

* Includes positive, negative and doubtful cases.
† The number of mice is given in parentheses.

The mechanism of this maternal transmission has not been demonstrated, but the probability is strong that the cytoplasm of the egg is involved since this constitutes the outstanding difference between the contribution of male and female germ cells. There remains, however, the possibility that some influence is transmitted through the placenta during gestation. The influence of mother's milk has been largely eliminated by fostering experiments in which C58 mice were nursed by foster mothers in nonleukemic strains and StoLi mice by C58 mothers without reducing the incidence of leukemia in C58 mice or inducing it in StoLi mice.

Whereas the expression of both leukemia and mammary carcinoma is subject to nonchromosomal influence transmitted by the mother, when this is eliminated there remain in each case other unidentified sources of nonchromosomal and entirely nongenetic influence the effectiveness of which is even greater.

10. Little, C. C.: Science **78**:465, 1933.

Although clearcut dominance and recessiveness have been called into the discussion of the manner of inheritance of leukemia by Slye,[4] it is certain that no such phenomenon is represented by these results. One might indeed consider that leukemia is a recessive trait stimulated into expression in hybrids by nongenetic variables, but one could equally well consider that it is a dominant trait the expression of which is inhibited by certain nonchromosomal variables. Thus the terms "dominance" and "recessiveness" have no meaning in this case. This conclusion agrees with an enormous mass of genetic evidence indicating that the concept of complete dominance has little if any general significance (Hogben[11]).

BACK CROSS TO STOLI

Just as breeding tests gave direct verification of the evidence that pure bred mice of strain C58 were genetically alike whether they had leukemia or not, the genetic uniformity of mice in the first hybrid gener-

TABLE 8.—*Breeding Tests of Hybrids (F_1) with Different Diagnosis* *

Diagnosis of Hybrid Parents	Offspring			Percentage of Definite Diagnoses Positive for Leukemia
+	87	87	10	50.0
−	16	21	7	43.2
?	21	27	12	43.8

* Pregeny of F_1 females from C58 mothers and StoLi fathers crossed back to StoLi males. The F_1 males used in the back cross were all leukemic.

ation has been directly substantiated by comparing the offspring of leukemic and nonleukemic hybrids crossed back to the pure bred strain StoLi. Table 8 gives the figures for this comparison.

Table 9 gives further evidence of nonchromosomal maternal influence. For in this back cross generation the incidence of leukemia is significantly higher (26.7 per cent) when the hybrid parent is the mother.

In back crosses genetic segregation occurs. Although the individual mice in the first hybrid generation are alike, the germ cells they produce are diverse, including all possible combinations of the genes by which the parent strains differ. So far as genetic constitution is directly expressed in the somatic conditions of the individual mice the back cross provides evidence of the number of genes by which the two pure strains differ. Thus the mice in this generation were divisible according to color of hair into eight equal groups corresponding to the eight possible combinations of the three pairs of genes influencing the color of hair by which C58 and StoLi differ. In the same way have occurred the segregation and recombination of all other genetic determiners,

11. Hogben, L.: Nature and Nurture, New York, W. W. Norton & Company, Inc., 1934.

whether for structural, physiologic or pathologic traits. In regard to leukemia it is obvious that the genetic constitution is not directly expressed somatically; a leukemic and a nonleukemic mouse may have the same constitution, while two leukemic mice may be genetically different.

Thus the incidence of leukemia in the back cross does not give a basis for determining the number of units responsible for the hereditary difference between C58 and StoLi. Aid in such a genetic analysis would be afforded by findings that some gene influencing the occurrence of leukemia was located in the same chromosome as a gene for color of hair. This linkage would be indicated by irregularity in the distribution of leukemic mice among the eight color classes. However, the classification of data in this way showed essential uniformity in the distribution of leukemia among the color classes.

TABLE 9.—*Incidence of Leukemia in Back Cross Mice Classified According to the Sex of the Hybrid Parent (Matings of StoLi with Hybrids from C58 Mothers and StoLi Fathers)*

Hybrid Parents	Offspring			Percentage of Definite Diagnoses Positive for Leukemia	Difference P.E.
	+	—	?		
Father	19	77	18	19.8	
Mother	74	85	29	46.5	
Difference				26.7 ± 3.8	7.0

Animals from the reciprocal matings of the back cross also differ significantly in longevity. The average length of life (559 days, 188 mice vs. 652.4 days, 114 mice) is 93.4 ± 13.9 days longer (6.7 times the probable error) when the mother is pure-bred StoLi. Thus in two hybrid generations longer life is associated with less leukemia; this is also the case in the two pure-bred strains. But this relationship is not directly causal because (1) the difference in length of life is shown by the leukemic as well as by the nonleukemic mice; (2) from pure StoLi fathers the first hybrid generation and back cross animals show almost exactly the same length of life as the pure StoLi animals, 545, 559 and 564 days, respectively, yet they give 61.9, 46.5 and 1.3 per cent leukemic mice; (3) first generation hybrids with StoLi mothers and back cross animals with StoLi fathers give nearly the same proportion of leukemic mice, but the average length of life differs by 124 days. In other words, leukemia transmitted through fathers gives midparental incidence in both hybrid generations, whereas greater longevity transmitted through fathers gives the same length of life in the two hybrid generations as in the pure-bred longer-living strain. Transmitted by mothers the longevity in the two hybrid generations is significantly greater than in the pure-bred longer-living strain.

The simple fact is that with the maternal factor barred the incidence of leukemia is reduced about half when the total heredity from strain C58 is reduced one half; when this is again reduced by one half by the back cross the incidence of leukemia is again reduced about half. The rôle of heredity is unquestionable and can be expressed in quantitative terms. This is the type of correlation between heredity and somatic traits observed by Galton; on the basis of such evidence a general statistical description of heredity called Galton's law was formulated. However, this takes no account of the transmission of traits in terms of genes. With results of this type many theoretical interpretations in terms of genes could be proposed, but the only possibility of obtaining evidence to distinguish between these interpretations lies in testing the genetic constitution of every individual mouse in the back cross by its transmission of leukemia to its offspring in a second back cross. Classified on such a basis the animals in the first back cross would reveal a genetic ratio capable of significant interpretation. Unfortunately the program for these experiments did not include such a second back cross, which will have to await a repetition of the original crossing.

COMMENT

Strains of mice characterized by spontaneous conditions similar to the leukemia in strain C58 have been reported, respectively, by Dobrovolskaïa-Zavadskaïa,[12] McCoy-Hill[13] and Slye.[4] The first mentioned strain, in comparison with various other strains in the same laboratory which together gave an incidence of 6 per cent, showed an incidence of 20 per cent (total number of mice, 143). The second strain had been inbred since 1920 under the direction of Dr. T. Brailsford Robertson; in a sample of 216 mice 134 (62 per cent) showed some form of lymphoid or myeloid hyperplasia. Of these, 50 per cent had lymphoid, 14 per cent myeloid, 23 per cent atypical or mixed types of hyperplasia, and the remainder had what was called Hodgkin's disease. Slye gave a pedigree mostly of brother and sister matings showing the causes of death in 152 mice, of which 36 (23.7 per cent) had some form of leukemic disease; however, in one branch the incidence was much higher. Four successive generations of this branch consisted of (1) 3 leukemic and 3 nonleukemic mice, (2) 5 leukemic and no nonleukemic mice, (3) 3 leukemic and no nonleukemic mice, and (4) 3 leukemic and no nonleukemic ones. Slye is the only author who deals directly with the genetics of mouse leukemia; she believes that all neoplastic conditions, including leukemia,

12. Dobrovolskaïa-Zavadskaïa, N.: Compt. rend. Soc. de biol. **109**:339, 1932.
13. McCoy-Hill, F.: J. Cancer Research **14**:325, 1930.

depend on a single recessive mendelian gene. However, geneticists do not concur in this opinion since the evidence necessary for such a conclusion has not been given.

Her case rests on the accumulation of instances: The paper on leukemia is based on 36 cases of leukemic diseases in one pedigree; 14 others are mentioned specifically, while 924 cases in other pedigrees in the first 50,000 autopsies are not accounted for in any way. No explanation is given of this abstemious use of available data, or of why this particular set was presented. Such selected evidence might justify a working hypothesis, but an acceptable conclusion requires the elimination of alternative hypotheses by experimental tests.

Although dealing with an experimental animal and using the terminology of genetics, Slye has not used the basic genetic test. Cross breeding is the critical tool for analysis of genetic differences. It consists of deliberate matings between animals from strains showing consistent differences; the generations designated as F_1 and F_2, etc., are defined by the experimental procedure; the results can be checked by repetitions with different animals. In contrast to this the procedure of Slye has been to breed the mice and after all were dead to decide from the results which mating was a "cross." The data presented concern an inbred pedigree of 11 generations originating in a single pair of mice with a "common house mouse" as father in one of the matings in the second generation (a mating that was not referred to as a "cross"). All other matings shown on the charts were between brother and sister. The failure to submit her hypothesis to the tests of a cross was not due to the lack of a contrasted strain, for she states: "There have been very many strains in the laboratory completely free from any form of these diseases. Into these strains I have never bred any leukemia nor has it arisen sporadically in such strains" (conclusion 2, p. 1384).

But before the number of genes can be determined by the segregation following a cross it is necessary to delimit the influence of nongenetic variables. Slye's inbred pedigree represents the progress of genetic purification which must precede an evaluation of nongenetic variables, but her data stop at the point at which such an evaluation might have begun. With the relative potency of genetic and nongenetic factors unestablished, any interpretation of her data must remain purely hypothetic. If nongenetic factors influence the results, as Slye has admitted in certain places and as the present study amply affirms, a single gene will not yield conventional ratios, and so the close similarity of certain of her results to mendelian ratios serves to deny the very hypothesis which she claims they verify.

Slye offers no interpretation of the genetic differences between strains in the frequency and in the type of neoplasm, although she is apparently dealing with such differences. She stresses the point that leukemia occurs in strains along with other tumors, but also states that she has tumor strains in which leukemia has never appeared. In spite of this indication that the genetic control of leukemia differs from that of other neoplasms, the genetic conclusions on leukemia are given only in terms of malignancy and nonmalignancy.

CONCLUSIONS

The predisposition to leukemia and related diseases in mice of strain C58 is specifically heritable.

In crosses with strain StoLi the incidence of leukemia is roughly correlated with the proportion of total heredity from strain C58.

In the incidence of leukemia nonchromosonal variables play a rôle that grows increasingly important as the proportion of total leukemic heredity is reduced.

Among nonchromosomal influences that act as deciding factors in the incidence of leukemia an important one is transmitted by the mother.

Under these conditions somatic expression of leukemia in a segregating generation cannot disclose genetic ratios. Such ratios can be obtained only by classification according to progeny tests.

THE NATURE OF THE ANEMIA IN ACUTE LEUKEMIA

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One of the most characteristic symptoms of acute leukemia is the severe and rapidly progressing anemia, which in the subleukemic and aleukemic form often causes considerable difficulties in differential diagnosis (Kracke,[1] Naegeli,[2] Parker Weber,[3] Rosenthal[4] and others). This anemia may develop during the course of the disease, or it may precede it for weeks or even months. In some cases the disease first presents itself under the clinical and hematologic picture of aplastic anemia or hemorrhagic aleukia. Either spontaneously or under the influence of antianemic treatment the anemic condition may improve temporarily, and the blood count may show practically normal values. The second attack usually is typically leukemic; the treatment that prompted the first remission is of no avail, and the disease terminates rapidly in death (Colarizi,[5] Klein,[6] Knudsen,[7] Scholtz,[8] Ullrich[9] and others). With the diagnosis of leukemia established, reexamination of the slides of the blood made during the first attack may reveal single very immature white blood cells, which at the first examination were mistaken for lymphocytes. These cases of severe initial anemia and leukopenia have induced some investigators to assume that there are pathogenic relations between the aleukias and leukemia, considering the two as different reactions to the same etiologic agent (Ederle and Esche,[10] Segerdahl,[11] Rittmann,[12] Strumia[13] and Ullrich[9]).

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1. Kracke, R. R., and Garver, H.: J. A. M. A. **104**:697, 1935.
2. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, ed. 5, Berlin, Julius Springer, 1931.
3. Weber, F. P.: Quart. J. Med. **1**:409, 1932. Weber, F. P., and Weisswange, W.: Deutsches Arch. f. klin. Med. **176**:422, 1934.
4. Rosenthal, N.: Am. J. Clin. Path. **1**:7, 1932.
5. Colarizi, A.: Haematologica **16**:49, 1935.
6. Klein, cited by Naegeli.[2]
7. Knudsen, O.: Ugesk. f. læger **96**:1374, 1934.
8. Scholtz, H. G.: Folia haemat. **45**:352, 1931.
9. Ullrich, O.: Ztschr. f. Kinderh. **53**:487, 1932.
10. Ederle, W., and Esche, G.: Folia haemat. **52**:179, 1934.
11. Segerdahl, E.: Folia haemat. **52**:68, 1934.
12. Rittmann, R.: Folia haemat. **51**:207, 1934.
13. Strumia, M. M.: Am. J. M. Sc. **187**:826, 1934.

At the present time the severe anemia of acute leukemia is generally attributed to the replacement of erythropoietic tissue by leukemic tissue. Whipple and Robscheit-Robbins,[14] who analyzed the liver chemically in a series of cases of acute leukemia, did not find an increase in the iron content and concluded that there was no or very little evidence of excessive destruction of the blood. On the other hand, von Kress [15] pointed to the hemosiderosis of the blood-forming organs and to the increased excretion of urobilin in the feces and in the urine as indicating an excessive destruction of erythrocytes. The hemorrhages, which are common in cases of acute leukemia, may contribute to the anemia, but there is no constant parallelism between the progression of the anemia and the intensity of the hemorrhagic diathesis. Reference has also been made to the possibility that hemotoxic substances may be produced by the leukemic tissue (Hirschfeld [16]). In an earlier paper [17] I described a marked hyperplasia of the erythropoietic tissue of the bone marrow and extramedullary foci of erythropoiesis in many cases of leukemia. I have also stressed the frequent occurrence of considerable hemosiderosis, suggesting that excessive destruction of the red blood cells rather than lack of erythropoietic tissue may account for the anemia. This conception is supported by the fact that in some cases of acute leukemia the patient dies of severe anemia before the leukemia has developed fully. Even though the blood picture may be typical of leukemia, microscopic examination of the organs shows only slight leukemic changes and a considerable amount of normal myelopoietic tissue. During the last three years I have observed five patients whose cases fit into this description, and since these cases may cast light on the obscure pathogenesis of acute leukemia, they are reported here in detail.

REPORT OF CASES

CASE 1.—*History and Course.*—A Negro boy aged 4 years was first admitted to a surgical ward because fracture of the skull was suspected. Three days before admission the child fell against a table and remained unconscious for ten minutes. Three days later the nose started to bleed, and vomiting followed. The next day the stools were black. The child was restless during the night and the next morning appeared drowsy.

On his admission the temperature was 98.8 F., the respiratory rate 28 and the pulse rate 170. The liver could be palpated 2 fingerbreadths below the costal arch, and the spleen extended for 2 fingerbreadths below the costal arch. In the

14. Whipple, G. H., and Robscheit-Robbins, F. S.: J. Exper. Med. **57**:671, 1933.

15. von Kress, H.: Deutsches Arch. f. klin. Med. **176**:359, 1934.

16. Hirschfeld, H., in Schittenhelm, A.: Handbuch der Krankheiten des Blutes, Berlin, Julius Springer, 1925, vol. 1.

17. Jaffé, R. H.: Folia haemat. **49**:51, 1933.

skin there were numerous hemorrhagic areas ranging from the size of a pinpoint to a diameter of 2 inches (5 cm.). The axillary and inguinal lymph nodes were palpable.

The Wassermann and Kahn reactions were negative. The blood showed 8.68 mg. of uric acid per hundred cubic centimeters. The urine was normal. Blood was noted in the stools.

Repeated intramuscular injections of whole blood had no effect, and the child died on the third day after admission, after an illness of apparently only six days.

Examination of the Blood.—The blood count showed: erythrocytes, 1,260,000; hemoglobin, 4.2 Gm. (Newcomer); reticulocytes, 4.8 per cent; leukocytes, 33,800; hemocytoblasts (stem cells), 27.2 per cent; large lymphocytes, 27.2 per cent; small lymphocytes, 19.2 per cent; promyelocytes, 0.8 per cent; metamyelocytes, 2.4 per cent; neurophilic leukocytes, 8 per cent; plasma cells, 0.8 per cent, and monocytes, 14.4 per cent. There were marked poikilocytosis and anisocytosis, many polychromatophils and 7 normoblasts and 1 erythrogonium, per hundred leukocytes. There were 82,000 platelets. The bleeding time was six and one-half minutes.

Gross Postmortem Examination.—There were evidences of severe anemia. Petechial hemorrhages were observed in the skin, lungs, pleura, epicardium, renal pelvis, brain and leptomeninges. There was fatty degeneration of the myocardium, liver and kidneys. The spleen was hyperplastic, weighing 110 Gm. and measuring 11 by 8 by 4 cm. The thymus weighed 12 Gm. Slight hyperplasia and deep purple-red discoloration of the peripheral, thoracic and abdominal lymph nodes were noted. The largest lymph node was 10 mm. in diameter.

Histologic Postmortem Examination.—The bone marrow (femur, sternum) was extremely congested with blood. There were only a few fat cells and the majority of the sinusoids and capillaries were collapsed, the blood cells being located outside the preformed blood spaces. Enclosed in the dense accumulations of erythrocytes were small islands of nucleated elements, and an occasional dilated sinusoid was filled with those cells. The nucleated elements occupied approximately 17 per cent of the marrow space. A differential count showed: undifferentiated lymphoid cells with a finely reticulated chromatin structure of the nucleus and a distinct rim of homogeneous basophilic cytoplasm (stem cells or hemocytoblasts), 11.4 per cent; myeloblasts, 0.8 per cent; neutrophilic myelocytes (the majority with poorly defined and disintegrating granulation), 8 per cent; neutrophilic leukocytes, 3 per cent; oxyphilic myelocytes, 8 per cent; oxyphilic leukocytes, 3.2 per cent; erythrogonia, 2.8 per cent; erythroblasts, 16 per cent; normoblasts (many very large ones with polychromatic or orthochromatic cytoplasm), 46.2 per cent; megakaryoblasts, 0.4 per cent; megakaryocytes (poorly preserved, with pyknotic nuclei and homogeneous cytoplasm), 1 per cent; lymphocytes, 1 per cent; plasma cells, 0.2 per cent; monocytoid cells, 2 per cent; free histiocytes with engulfed red blood corpuscles, 3 per cent.

The liver, lymph nodes and especially the spleen revealed marked myeloid metaplasia. In the spleen, which was greatly congested, erythropoiesis predominated. The liver showed erythropoiesis and granulopoiesis in and about the portal sinusoids and in the periportal septums. The lymph nodes contained neutrophilic and oxyphilic myelocytes and megakaryocytes. In addition to the myeloid cells which showed the tendency to mature and did not differ from the cells seen in cases of reactive extramedullary myelopoiesis, there were groups and nests of undifferentiated cells which were identical with those described in the

bone marrow. They gave a negative reaction for oxidase and could be readily differentiated from lymphocytic elements as well as from myeloblasts, which give a positive reaction for oxidase. They were more numerous in the liver than in the spleen and were scanty in the medullary cords of the lymph nodes. The purple-red discoloration of the lymph nodes was due to the filling of the sinusoids with red blood corpuscles, many of which had been taken up by the swollen and proliferated reticular endothelium of the sinusoids. There was also a moderate erythrophagocytosis by the Kupffer cells of the liver. In the medulla of the lymph nodes the small lymphocytes showed a peculiar crenation and segmentation of the nuclei, which was also found in the small round cells of the thymus. The lymphatic tissue of the pharynx and intestinal tract was normal. The kidneys, lungs and glands of internal secretion were normal. In the brain the hemorrhages were located about small blood vessels. Between the walls of the vessels and the hemorrhages there was often a zone of lymphoid round cells or a zone of necrosis with swollen microglia cells and occasional lymphoid cells.

The reaction for iron was as follows: Kupffer cells, +++; hepatic cells, +; splenic pulp, ++; lymph nodes, +++; bone marrow, +, and kidney, 0.

CASE 2.—*History and Course.*—A white woman, a housewife aged 38, for the past seven weeks had been suffering from frequent vomiting. The vomitus consisted of bright red blood or of coffee-ground material. The medicine prescribed by a physician had no effect. Occasionally there was also bleeding from the gums. A few days before admission the patient started to cough and became severely constipated. Epigastric distress developed, but there was no selective dyspepsia or any relation to food. Since childhood the patient had bruised easily. She had had three normal deliveries. Pneumonia developed several years ago. Pelvic laparotomy was performed seventeen years prior to admission.

On admission the temperature was 100 F., the pulse rate 112 and the respiratory rate 28. The blood pressure was 130 systolic and 90 diastolic. The patient was pale, and the tongue and gums were covered with a film of loosely coagulated blood. There were retinal hemorrhages in the right eye. The teeth were in poor condition. The tonsils were moderately enlarged. Over the apex of the heart a soft systolic murmur was heard. The lower pole of the spleen could be felt below the costal arch. Over the extremities there were scattered petechiae, and around wounds from needle punctures large ecchymoses had developed.

The urine was normal. The Wassermann and Kahn reactions were negative. Roentgen examination of the gastro-intestinal tract revealed no abnormalities.

During the patient's stay in the hospital the temperature rose at times to 105 F. The hematemesis persisted, and she became rapidly weaker. She expired five days after admission to the hospital after an illness of about seven weeks. Two blood cultures were sterile.

Examination of the Blood.—The blood count showed; erythrocytes, 1,800,000; hemoglobin (Sahli), 22 per cent; leukocytes, 10,600; neutrophilic myelocytes, 3 per cent; juvenile neutrophilic leukocytes, 4 per cent; band forms, 10 per cent; segmented forms, 37 per cent; lymphocytes, 30 per cent; monocytes, 15 per cent, and basophils, 1 per cent. The neutrophilic granulocytes were distinctly smaller than normal. Some of them were greatly shrunken. The granulation was abnormal. Many cells had a deeply stained granulation, while in some of the cells the granules were transformed into rose-red droplets. The monocytes had a heavy azure granulation. Marked anisocytosis and poikilocytosis and single hyperchromatic and polychromatic macrocytes were noted. There were 5 normoblasts per hundred leukocytes. There were 40,000 platelets.

Gross Postmortem Examination.—Petechial hemorrhages in the skin, epicardium, endocardium, gastric mucosa, urinary bladder and renal pelvis and moderate hyperplasia of the spleen (weight, 410 Gm.; size, 19 by 10 by 5.5 cm.) were noted. The liver weighed 1,660 Gm.; the heart, 310 Gm. Hemosiderosis of the spleen, liver, bone marrow and lymph nodes, slight hyperplasia of the peripheral, thoracic and abdominal lymph nodes (greatest diameter, 15 mm.) and confluent bronchopneumonia in the lower lobe of the left lung were noted.

Histologic Postmortem Examination.—The bone marrow (femur) was rich in red blood corpuscles, which were found chiefly outside the capillaries and sinusoids. The capillaries and sinusoids were collapsed, and fat cells were numerous. The reticular cells were prominent and contained many red blood cells. Immature blood cells occupied about 19.6 per cent of the marrow. A differential count showed 17 per cent undifferentiated lymphoid cells, from three to five times the size of small lymphocytes with a distinct rim of homogeneous basophilic cytoplasm that gave a negative reaction for oxidase and contained round nuclei with finely reticulated chromatin of varying density. The count also showed myeloblasts, 0.3 per cent; neutrophilic myelocytes, 19.8 per cent; neutrophilic leukocytes, 6.6 per cent; eosinophilic myelocytes, 0.5 per cent; eosinophilic leukocytes, 0.2 per cent; erythrogonia, 9.3 per cent; erythroblasts, 8.1 per cent; normoblasts (some of them very large with bizarre-shaped, pyknotic nuclei), 34.9 per cent; lymphocytes, 0.8 per cent; plasma cells, 0.2 per cent; monocytoid cells, 1.6 per cent; megakaryoblasts, 0.1 per cent, and megakaryocytes, 0.5 per cent.

The spleen showed extensive myeloid metaplasia of the pulp, with numerous myeloblasts, neurophilic and oxyphilic myelocytes, many erythroblasts and normoblasts and single megakaryocytes. The malpighian bodies were much reduced in size. In the liver a moderate number of myeloblasts, myelocytes and erythroblasts were observed in the lumen of the sinusoids and in the periportal tissue. There were also a few megakaryocytes. The Kupffer cells were actively phagocytic. All the lymph nodes revealed advanced myeloid metaplasia, and nests of myeloid cells were also present in the fat tissue about the lymph nodes. The medulla and the cords between the cortical nodules were composed of myeloblasts, myelocytes, erythroblasts, normoblasts and single megakaryocytes. There was a great deal of erythrophagocytosis by the proliferated reticular endothelium of the sinusoids. The tracheobronchial and, in particular, the abdominal, lymph nodes contained also nests of undifferentiated lymphoid cells, which often formed a syncytium. These nests sometimes revealed intimate relations to the cytoplasmatic reticulum of the node. The thyroid gland, adrenal glands, pancreas, kidneys, heart and gastro-intestinal tract showed no significant changes.

The reaction for iron was as follows: Kupffer cells, +++; hepatic cells, 0; splenic pulp, +++; malpighian bodies, ±; lymph nodes, +++; bone marrow, +++, and kidney, +.

Case 3.—*History and Course.*—For the past seven months a Negro boy aged 11 years had been suffering from remittant pains in the hips, occasionally also of the knees and shoulders. The pains lasted for about two weeks and confined him to bed. On the day of his admittance to the hospital severe epistaxis had occurred. The boy had whooping cough and measles in infancy. There was unexplained fever for two weeks two years previously, and acute tonsillopharyngitis developed two months prior to admission.

The child was very pale and weak. The nose was filled with crusted blood, and he vomited everything he ate. The temperature was 98, the pulse rate 128

and the respiratory rate 24. The spleen was not palpable, and the peripheral lymph nodes were not enlarged.

The Wassermann and Kahn reactions were negative. Two blood cultures gave negative results. The urine showed a trace of albumin. The stools were normal. Chemical analysis of the blood showed urea nitrogen, 28.45 mg., creatinine, 1.96 mg., and sugar, 106 mg., per hundred cubic centimeters. Roentgen examination showed osteoporotic changes in both tibias and slight sclerosis of the ilium but no changes in the skull.

Three days after admission a blood transfusion was given. Five days later the temperature rose to 103 F. The next day a second transfusion was given. Lymph nodes ranging in size from that of a pea to that of a filbert could then be palpated in both axillae and both groins.

A lymph node was removed from the right groin for biopsy, and the examination showed hyperplasia, the tissue between the secondary nodules of the cortex and the cords of the medulla being infiltrated by undifferentiated lymphoid cells, larger than lymphocytes, with finely reticulated nuclei and scanty cytoplasm, and by single megakaryocytes. The picture was suggestive of an early stage of hemocytoblastic leukemia.

A few days later a serpiginous ulcer developed at the right side of the mouth and gradually spread to the right cheek. By the seventeenth day the spleen was distinctly palpable at the costal arch, having increased rapidly in size. The temperature varied between 101 and 102 F. Roentgen treatment was given to the upper mediastinum. The child died four weeks after admission to the hospital and eighteen days after the last blood transfusion.

Examination of the Blood.—Blood counts were made every third day. On the day of admission the erythrocytes numbered 1,330,000, the hemoglobin content (Sahli) was 35 per cent and the leukocyte count was 14,550. During the following weeks the number of erythrocytes gradually fell to 600,000, the hemoglobin content to 15 per cent and the leukocyte count to 1,800. There were from 30 to 44 per cent undifferentiated cells (hemocytoblasts). These cells were two or three times the size of small lymphocytes. The nucleus was round, with a deep, narrow indentation, which became marked toward the end of the illness. The nucleus contained a dense, fine net of small chromatin granules which obscured the nucleoli. The cytoplasm was narrow; it stained bright blue with Wright's stain and showed a negative reaction for oxidase. At times 4 per cent of the cells showed mitotic division of the nucleus with about twenty-four chromosomes. There were from 5 to 44 per cent neutrophilic leukocytes, the rest being small lymphocytes. There were marked anisocytosis and poikilocytosis, very few polychromatophils, an average of 0.2 per cent of reticulocytes and 170,000 platelets. The bleeding time was six minutes; the coagulation time, four minutes.

Gross Postmortem Examination.—Examination showed: gangrenous stomatitis; osteoporosis, and petechial hemorrhages in the conjunctiva of the right eye, epicardium, gastric mucosa, Peyer's patches, kidneys and renal pelvis. There was slight hyperplasia of the spleen (120 Gm.). The liver weighed 920 Gm. Fatty degeneration of the myocardium and moderate enlargement of the peripheral, thoracic and abdominal lymph nodes (greatest diameter, 20 mm.) were noted.

Histologic Postmortem Examination.—The cortex of the long bones was unusually thin, and the haversian canals were very wide. The trabeculae of the spongiosa were far apart and slender. In the skull the diploe was compact, and the marrow spaces were narrow. The bone marrow was extremely hemorrhagic (fig. 1), looking like a lake of blood crossed by an occasional compressed capillary.

Here and there a small nest of undifferentiated lymphoid cells and single normoblasts, neutrophilic myelocytes and megakaryocytes were found. The reticular cells were swollen and contained many engulfed erythrocytes. Similarly, the pulp of the spleen was greatly congested with blood. About the sheathed arteries, the trabeculae and the malpighian bodies there were accumulations of undifferentiated lymphoid cells which gave a negative reaction for oxidase and were often so densely grouped together that they seemed to form a syncytium. Bordering at the pulp, myeloblasts, hematogonia, erythroblasts, normoblasts, neutrophilic and oxyphilic myelocytes and plasma cells were found between the lymphoid cells. The undifferentiated cells were distinctly different from the small lymphocytes of the malpighian bodies. Many of the reticular cells of the pulp were filled with

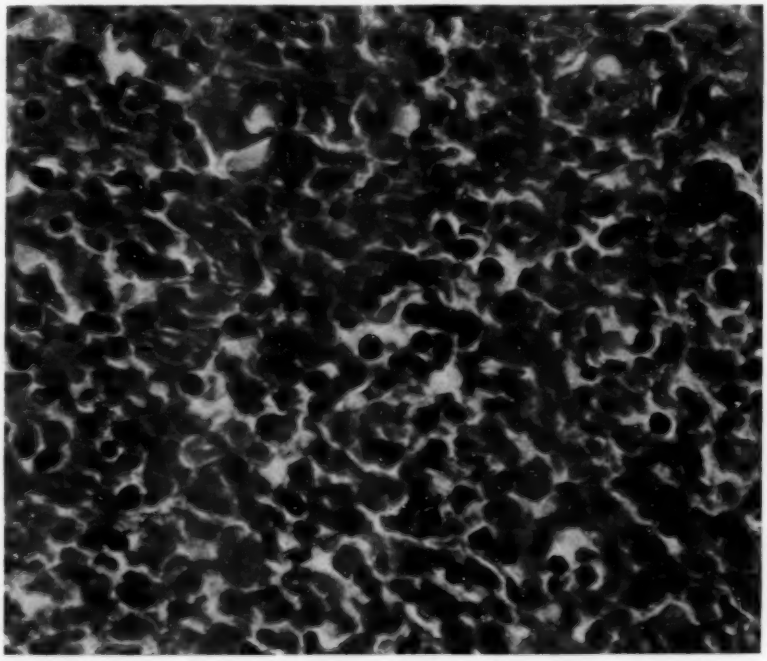

Fig. 1 (case 3).—Hemorrhagic bone marrow (× 600).

erythrocytes and nuclear débris. The portal sinusoids of the liver contained a moderate number of undifferentiated lymphoid cells, myeloblasts, normoblasts, neutrophilic myelocytes and a few mature neutrophilic leukocytes. The periportal tissue was densely infiltrated by the cells described as present in the lumens of the sinusoids. The Kupffer cells were prominent and contained erythrocytes and nucleated cells.

In the lymph nodes the enormous erythrophagocytosis by the proliferated endothelial cells of the sinuses was striking (fig. 2). The cortex and medulla were rich in undifferentiated lymphoid cells, and there were also many myeloblasts and normoblasts and a few neutrophilic and oxyphilic myelocytes and large plasma cells. The para-aortic nodes contained many megakaryocytes. In the mediastinal and mesenteric nodes the cortical secondary nodules were well preserved and

showed histiocytic centers. About the larger vessels of the kidney, especially at the border of the cortex and the medulla, there were dense accumulations of undifferentiated cells which extended between the adjacent tubules. They were mixed with plasma cells, normoblasts and neutrophilic myelocytes.

The reaction for iron was as follows: Kupffer cells, +; hepatic cells, ±; spleen, +++; lymph nodes, +++; bone marrow, +, and kidney, 0.

CASE 4.—*History and Course.*—The patient was a white man, aged 30, who had been employed as a color mixer in a soap factory. He stated that he was well until eleven weeks prior to admission, when a sharp, shooting pain developed at the tip of the spine and radiated toward the shoulders. At times there was pain in all the joints. He had lost a good deal of weight and felt exceedingly weak. One month prior to admission he began to cough up blood and to bleed from the gums.

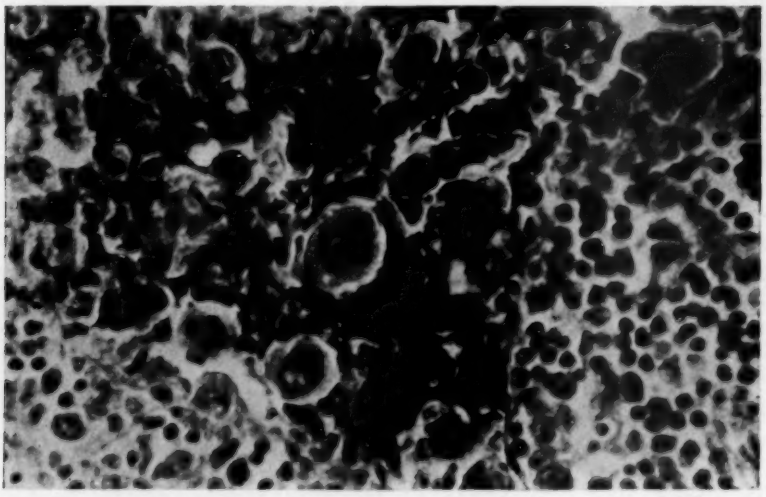

Fig. 2 (case 3).—Erythrophagocytosis by the sinus endothelium of an iliac lymph node (× 600).

On admission the patient was very pale. The posterior cervical and the axillary lymph nodes were slightly enlarged, discrete and firm. The heart and lungs were normal. The lower border of the liver was 2 fingerbreadths below the costal arch, and the lower pole of the spleen could be easily palpated. The spleen felt firm. There was a moderate enlargement of the epitrochlear and inguinal lymph nodes.

The icterus index was 3.5. The urine was normal. The temperature was 98.4 F.

A biopsy specimen from an inguinal lymph node revealed marked myeloid metaplasia with predominance of very immature hemocytoblastic cells.

During his stay in the hospital the patient became more anemic, and purpuric spots appeared over the extremities. A systolic murmur became audible over the apex of the heart. Two weeks prior to death he complained of failing vision, and an ophthalmoscopic examination disclosed retinal hemorrhages. He bled from the mouth and nose and died three weeks after admission to the hospital.

Examination of the Blood.—On the day of admission the blood showed: hemoglobin (Sahli), 30 per cent; erythrocytes, 1,920,000, and leukocytes, 4,750. The undifferentiated cells, which predominated, varied greatly in size, the smallest forms being slightly larger than small lymphocytes, while the largest cells reached a diameter of 30 microns. They were round or short oval, occasionally with semispherical, pseudopodia-like protrusions. The cytoplasm was scanty and basophilic and often contained several small vacuoles. The reaction for oxidase was negative. The nucleus occupied from two thirds to four fifths of the cell body and was round or oval with small indentations. The chromatin structure was densely reticular, and no nucleoli were seen. A differential count showed: undifferentiated cells, 84 per cent; small lymphocytes, 8 per cent; neutrophilic leukocytes, 4 per cent; monocytes, 4 per cent, and blood platelets, 14,000. During the course of the illness the hemoglobin value decreased to 20 per cent, and the number of erythrocytes, to 1,140,000. The number of leukocytes fluctuated between 4,000 and 22,000 and shortly before death reached 5,100. The undifferentiated cells increased to 90 per cent.

Gross Postmortem Examination.—Petechial hemorrhages were present in the mucosa of the lips, stomach and small intestine, the epicardium, endocardium and myocardium and the mucosa of the urinary bladder. The organs were severely anemic. There was marked hyperplasia of the spleen (weight, 1,015 Gm.; size, 23 by 13 by 8 cm.). The liver weighed 2,550 Gm. There were: moderate generalized lymphadenopathy, the largest nodes having a diameter of 30 mm.; fatty changes of the myocardium; centrolobular focal necrosis of the liver; hemosiderosis of the liver and spleen, and an old anemic infarct in the lower lobe of the right lung.

Histologic Postmortem Examination.—The bone marrow (femur and rib) was composed of fat tissue with many focal, extravascular accumulations of blood. There were small islands of cells, occupying about 20 per cent of the narrow, which were formed by round cells the scanty cytoplasm of which was free from oxidase granules and often revealed small vacuoles. The nuclei contained a dense, fine net of small chromatin granules. In some of the cells the nuclei were indented, and the indentation was associated with a more compact chromatin structure. Occasionally, a nucleus was divided into two or three lobules. There were a few orthochromatic normoblasts, but no granulated cells were observed. In the pulp of the spleen, in the periportal tissue of the liver, extending into the adjacent portions of the lobules, in the peripheral, thoracic and abdominal lymph nodes and in the stroma of the kidneys, there were dense accumulations of cells similar to those noted in the bone marrow and typical of the condition. The nuclei showed the same indentations and segmentations, and the cells could be well separated from the lymphocytes. The reaction for oxidase was negative. In the lymph nodes many mitotic figures were seen. The portal sinusoids of the liver contained many typical cells. Layers of typical cells were found underneath the endothelium of the splenic, portal and pulmonary veins (fig. 3). Small groups of typical cells were also present in the interstitial tissue of the myocardium and about the lymphatic tissue of the intestinal tract. The arteries and veins leading to the infarct of the lung were occluded by organized thrombi, and in the granulation tissue filling the lumen there were groups of the undifferentiated round cells. In addition to the typical cells a moderate number of normoblasts and neutrophilic and oxyphilic myelocytes were observed in the splenic pulp, the liver and the lymph nodes. The inner zone of the adrenal cortex contained groups of normoblasts. A striking feature was the large number of plasma cells in all the hematopoietic organs. These cells reached a large size, and many of them had two nuclei.

The reaction for iron was as follows: Kupffer cells, +++; hepatic cells, +++; spleen, +++; lymph nodes, +; kidney, ±, and bone marrow, 0.

CASE 5.—*History and Course.*—At the age of 5 years the patient, a white girl, had slight swelling and redness of the nose; this disappeared rapidly but recurred a year later. It again disappeared without treatment after a short time. Several months later it recurred again and then continued to grow slowly until it reached the size of a small egg. It closed the right eye and pushed the nose toward the left and the right side of the mouth downward. The swelling was firm, with a smooth, shiny, purple-brown surface. The surrounding skin was unchanged. Except for that lesion the child seemed to be in perfect health. A specimen was taken for biopsy. Ten radium treatments were given in the course of eight months.

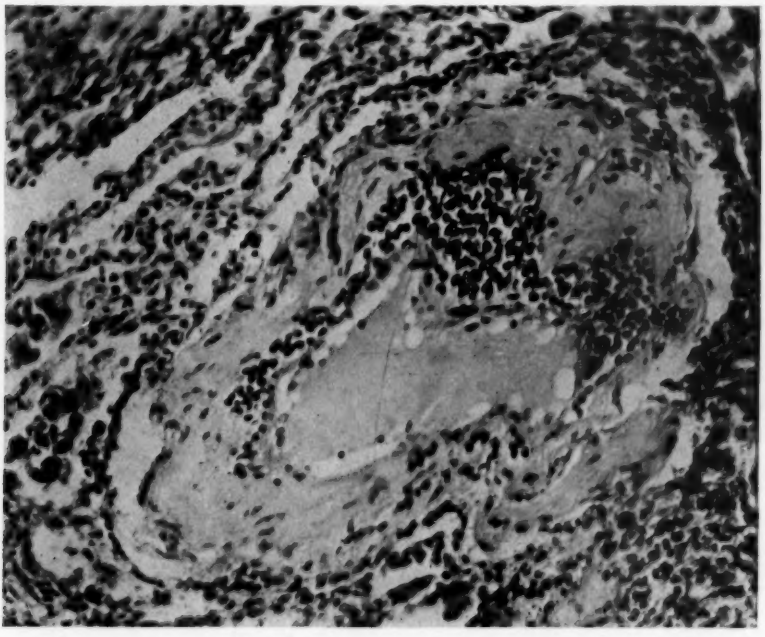

Fig. 3 (case 4).—Subendothelial proliferation of undifferentiated hematic cells (hemocytoblasts) in a small pulmonary vein (× 300).

The swelling disappeared gradually, and two months later there was only a small scar from the wound made in removal of the tissue for biopsy. Ten months later the child, then 8 years of age, had bloody diarrhea. The condition improved slightly, but the child felt extremely weak and feverish and complained of pains in the ears and of a cough. One week later she was admitted to the hospital.

On admission the child was pale. The temperature was 104.4 F., the pulse rate 154 and the respiratory rate 28. The drum of the left ear was deeply injected and thickened. The tonsils were enlarged and cryptic, and on the left side of the neck small, discrete lymph nodes could be felt. Fine, subcrepitant râles and broncho-vesicular breathing were audible over the left lung posteriorly, and a loud, blowing systolic murmur was heard over the apex of the heart. The liver extended 2 fingerbreadths below the costal arch, and there was tenderness on deep palpation

in the region of the spleen and the left kidney. The right axillary and both inguinal lymph nodes were enlarged and tender.

The urine was normal.

A blood transfusion was given, but the child died four days after admission.

Biopsy.—The epidermis was thin, and the rete pegs were flattened (fig. 4). The reticular layer of the cutis and the subcutis were densely infiltrated by round cells, and these infiltrations were sharply separated from the epidermis by a layer of intact dermis. The infiltrations surrounded the hair follicles and sweat glands without invading or disfiguring them. They were composed of medium-sized round cells of even size and structure with a scanty cytoplasm and round or oval nuclei. The nuclei were rich in chromatin, which formed small granules and was evenly distributed. Some of the nuclei were pyknotic and crenated. There were single cells which were much larger than the average cells and possessed a lobulated nucleus. There were numerous mitotic figures. The cells were embedded in a delicate reticulum. In places where the infiltrations were less compact, strands of hyalinized collagenous fibers were seen between them. At the periphery of the infiltrations small groups of plasma cells were seen. The diagnosis was leukemia cutis.

Examination of the Blood.—A blood count showed: hemoglobin, (Sahli) 17 per cent; erythrocytes, 650,000; leukocytes, 1,600; lymphocytes, 99 per cent; neutrophils, 1 per cent, and platelets, 350,000.

Gross Postmortem Examination.—The patient was severely anemic. There were petechial hemorrhages in the epicardium and endocardium; extensive hemorrhages in the mucosa of the colon; hemosiderosis of the liver, spleen, lymph nodes and bone marrow; severe fatty degeneration of the myocardium; fatty changes and centrolobular focal necrosis of the liver; tarry intestinal content; bronchopneumonia in the lower lobe of each lung, and a small scar on the right cheek. Cultures of the spleen and of blood from the heart were sterile.

Histologic Postmortem Examination.—The bone marrow of the femur and of the vertebral bodies was composed of a fine, loose reticulum the meshes of which contained red blood cells and single immature red cells and granulocytes. The erythrocytes varied considerably in size and in hemoglobin content, and many of them were pale. The nucleated erythrocytes showed similar variations. There was a bizarre and abnormal segmentation of the nuclei of the normoblasts, and the few erythroblasts also revealed segmentation of the nuclei. The granulation of the myelocytes was poorly preserved, and the granules were often transformed into droplets. Many large, free histiocytes were present, and their cytoplasm was filled with iron granules or stained diffusely blue in the sections tested for iron. The myeloid elements occupied about 6 per cent of the marrow. A differential count showed: neutrophilic myelocytes, 12 per cent; eosinophilic leukocytes, 1.6 per cent; erythroblasts, 4.8 per cent; normoblasts, 73.6 per cent; lymphocytes, 2 per cent; plasma cells, 2.4 per cent, and megakaryocytes, very scanty. The nuclei were pyknotic and the cytoplasm, homogeneous.

The splenic pulp was much congested with blood and rich in myeloid elements, among which normoblasts and neutrophilic and oxyphilic myelocytes predominated. While the granulation of the oxyphilic myelocytes stained well, that of the neutrophilic myelocytes was often indistinct. There were many plasma cells and single erythroblasts, myeloblasts and megakaryocytes. The portal sinusoids of the liver were filled with normoblasts, neutrophilic and oxyphilic myelocytes and myeloblasts. The myeloblasts formed small nests which were intimately connected with the cytoplasmatic reticulum formed by the Kupffer cells. The periportal

tissue was infiltrated by lymphocytes and contained also accumulations of myeloid cells. In the lymph nodes the sinuses were greatly widened and were filled by proliferated reticular endothelial cells which displaced active erythrophagocytosis. The sinuses as well as the cords of the medulla contained also neutrophilic and oxyphilic myelocytes, normoblasts and megakaryocytes. In the medullary cords of the para-aortic lymph nodes small nests of undifferentiated round cells were present which could be plainly distinguished from the lymphocytic cells and usually formed syncytial aggregates. In the myocardium the interstitial tissue was more cellular than normal owing to an increase in the myelocytes. In the fat tissue about the adrenal glands nests of mononuclear cells with ample cytoplasm were observed.

The reaction for iron was as follows: Kupffer cells, +++; hepatic cells, ±; spleen, +++; bone marrow, ++; lymph nodes, ++, and kidney, 0.

COMMENT

In the five cases described there were many significant similarities. In each case the illness was of relatively short duration, and there was extremely severe anemia with a hemorrhagic diathesis. The autopsy observations in four cases were not suggestive of leukemia, although in three of these cases the blood picture was definitely leukemic. The essential macroscopic observations were those of severe anemia and of an excessive destruction of blood, as indicated by the rusty discoloration of the liver, spleen and lymph nodes. Microscopically, the extensive destruction of the erythrocytes was one of the most striking features. In four cases the reticulohistiocytic cells of the blood-forming organs were packed with erythrocytes, and in all five cases there was marked hemosiderosis of the organs of the reticulohistiocytic system. Since erythrophagocytosis and hemosiderosis were present also in the cases in which blood transfusions had not been given, the excessive destruction of blood could not be related to the transfusions. Phagocytosis and disintegration of the red blood cells were also observed in lymph nodes that drained from areas which were not the site of hemorrhages. The excessive destruction of blood was associated with an increased reactive myelopoiesis which occurred chiefly outside the bone marrow. This extramedullary myelopoiesis was orderly and showed a distinct tendency to produce mature blood cells, not being different from the extramedullary formation of blood cells often observed in cases of severe anemia. The condition of the bone marrow was of great interest since it was different from the condition which is usually observed in cases of acute leukemia. The bone marrow was engorged with erythrocytes and revealed very little evidence of activity. In none of the five cases did the myelopoietic tissue occupy more than 20 per cent of the marrow spaces.

Recently puncture of the bone marrow has come into vogue, and great significance has been attributed to the examination of material obtained from the sternal marrow either by surgical removal or by

aspiration. If, for instance, in case 1, in which there was a distinctly leukemic blood picture, the sternal marrow had been examined in vivo, the result would have been disappointing. In case 2, in which the diagnosis was clinically obscure, sternal puncture probably would have been of little help in determining the correct diagnosis.

In addition to the typical reactive myelopoiesis in all five cases a proliferation of very immature blood cells was noted which varied in extent, being insignificant in case 5 and most marked in case 4. That type of proliferation of immature blood cells and their differentiation from the mesenchyme throughout the body occurs only in cases of leukemia. There is still much controversy as to the type of cell observed in certain cases of acute leukemia. The majority of investigators agree that in addition to myeloblastic, myelocytic, lymphoblastic, lymphocytic, monocytic and plasmacellular leukemia there are forms of leukemia which defy classification because the cells are so immature that they cannot be identified with any of the known precursors of white or red blood cells. There is growing evidence in favor of considering these very immature cells as the common parental cells of all blood cells. In the German literature the term stem cell leukemia (Hoff [18]) has been widely adopted, while Italian authors, under the influence of Ferrata, speak of hemocytoblastic leukemia (Marziani,[19] Debiasi,[20] Callerio,[21] Samek,[22] Simonetti [23] and many others). In this country both terms—hemocytoblastic leukemia and stem cell leukemia—are being used. Concerning the morphology of the hemocytoblast the reader is referred to the description given in the case reports. The hemocytoblast is a cell which gives a negative reaction for oxidase and which is distinctly different from the lymphoblast, monoblast, myeloblast, megakaryoblast and erythrogonium (proerythroblast, megaloblast). Showers of hemocytoblasts may enter the blood in any type of case of acute leukemia, and even in cases of subacute or chronic leukemia there may be a transient hemocytoblastic stage. In the earlier literature, published before the hemocytoblast had been recognized, the appearance of hemocytoblasts in a case of myelogenous leukemia was interpreted as a shift from the myeloid to the lymphatic side (mixed cell leukemia). Hemocytoblastic leukemia may take a leukemic, subleukemic or aleukemic course and in my experience has been the most common form of pure acute leukemia, the lymphatic and myelogenous form frequently being the terminal stage of chronic leukemia.

18. Hoff, F.: Virchows Archiv. f. path. Anat. **261**:142, 1926.
19. Marziani, R.: Arch. ital. di anat. e istol. pat. **1**:1015, 1930.
20. Debiasi, E.: Haematologica **12**:719, 1931.
21. Callerio, G.: Haematologica **13**:49, 1932.
22. Samek, E.: Haematologica **14**:37, 1933.
23. Simonetti, R.: Riv. di clin. pediat. **30**:161, 1932.

In three of my cases the hemocytoblast was found in the blood. The leukocyte count varied between 1,800 and 33,800. In other cases of hemocytoblastic leukemia which are not included in this report I have encountered leukocyte counts up to 700,000. In two cases there were also young granulocytes and precursors of erythrocytes in the blood smears, and in one case the granulocytes showed severe toxic alterations. In four cases the number of platelets was moderately to markedly diminished, while in one case it was within normal limits.

Case 5 is of particular interest. Three years prior to death a recurrent cutaneous lesion of the face developed which disappeared spontaneously twice within two years. With the third recurrence the lesion continued to develop and receded promptly under radium treatment. The child was in good health for ten months after the treatment had been completed. The clinical and histologic picture of the third recurrence was characteristic of the tumor-like form of specific leukemia cutis. I refer to the location at the root of the nose, to the sharp demarcation of the tumor-like lesion from the surrounding normal skin, to the arrangement of the infiltrating cells about the hair follicles and the sweat glands and to the distinct demarcation of the infiltrations from the subpapillary layer of the cutis, which was unchanged while the cells extended deep into the subcutaneous tissue (fig. 4; Arndt,[24] Gans,[25] Patrassi [26] and others). It is chiefly in the lymphatic form of leukemia cutis that the face is involved, especially the region about the nose (Gans,[25] Kreibich,[27] Hirschfeld,[16] Arzt [28]), and Pinkus [29] therefore spoke of the "acrolesions" in cases of lymphatic leukemia of the skin. From the classic form of lymphadenosis cutis the picture in my case differed slightly. The infiltrations were more pleomorphic, and the cells were larger than those that usually are found in lymphatic-leukemic infiltrations of the skin. Unfortunately I have no information in that case as to the blood picture during the stage of isolated leukemia cutis. There was, however, nothing in the clinical picture to suggest a blood dyscrasia.

Cases of isolated leukemia of the skin have been repeatedly described (Zumbusch,[30] Pinkus,[29] Roessle [31]), and even at autopsy the leukemic

24. Arndt, cited by Arzt.[28]

25. Gans, O.: Histologie der Hautkrankheiten, Berlin, Julius Springer, 1925, vol. 1, p. 575.

26. Patrassi, G.: Folia haemat. **50**:415, 1933.

27. Kreibich, K.: Arch. f. Dermat. u. Syph. **47**:185, 1899.

28. Arzt, L.: Wien. klin. Wchnschr. **46**:1125, 1933.

29. Pinkus, F.: Arch. f. Dermat. u. Syph. **50**:37, 1899.

30. Zumbusch, cited by Roessle.[31]

31. Roessle, R.: Virchows Arch. f. path. Anat. **275**:310, 1930.

process may be found to be almost exclusively restricted to the skin (Roessle [31]). In this connection the case described by Dragisic [32] may be cited. The patient, a girl aged 11 years, suffered from an aleukemic, leukopenic lymphadenosis, which after five months terminated in acute leukemia. Following roentgen treatment of the enlarged lymph nodes leukemic infiltrations of the skin developed. Later the infiltrations of the skin disappeared, while the lymph nodes enlarged again.

During the short final illness in my case there were very severe anemia and leukopenia with extreme neutropenia. The normal platelet

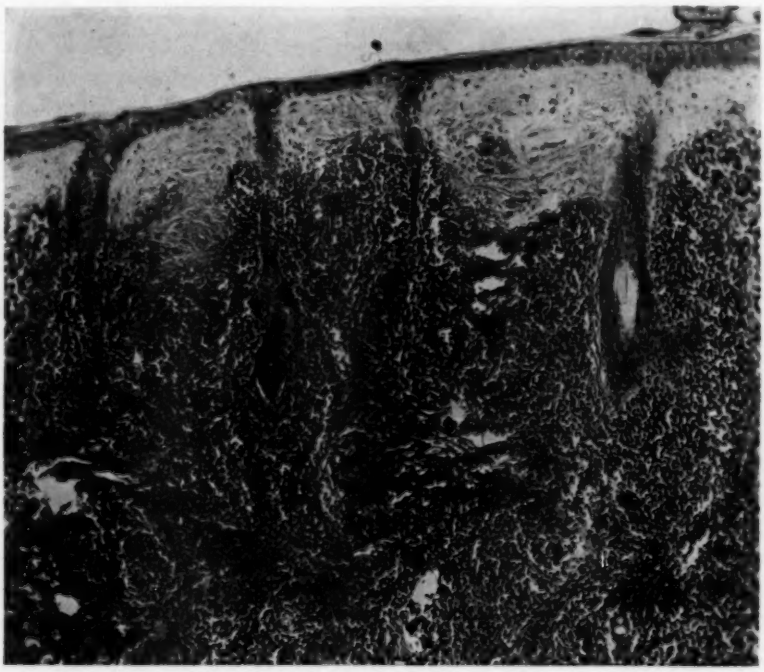

Fig. 4 (case 5).—Section of biopsy material from the skin, showing the arrangement of the infiltrations about the hair follicles and the sharp demarcation of the infiltrations from the subpapillary layer of the cutis, the stretching of the epidermis and the extension of the infiltrations into the subcutis (× 150).

count spoke against a diagnosis of aplastic anemia or hemorrhagic aleukia. Anatomically the leukemic changes were very insignificant and consisted of a focal proliferation of very immature hematic cells in the medullary cords of the para-aortic lymph nodes. By comparing the changes in the para-aortic lymph nodes with those seen in cases of typical hemocytoblastic leukemia, one can readily recognize that the

32. Dragisic, B.: Wien. klin. Wchnschr. **45**:1165, 1932.

differences were merely quantitative and not qualitative. The chief argument in favor of the diagnosis of very early acute leukemia was the preceding tumor-like leukemic infiltration of the skin of the face. I am therefore of the opinion that the case was one of isolated leukemia cutis in which almost one year after the leukemic lesion of the skin had been treated successfully with radium death occurred in the initial anemic stage of acute leukemia. Because of the long interval between the radium treatment and the onset of anemia, causal relations between the two can be excluded.

CONCLUSIONS

The early stages of a disease offer the best possibility to study the pathogenesis, since in the later stages the changes may have advanced so far that they obscure the underlying pathologic process. Clinical and experimental observations have repeatedly suggested that leukemia may be related to or follow an abnormal destruction of blood. Thus leukemia has been observed in persons exposed to benzene, arsenicals, radium and other substances known to cause destruction of the blood cells (Emile-Weil [33]; Deloré and Bergomano [34]; Falconer [35]; Vaughan, Terplan and Sanes,[36] and others). Leukemia has been produced in mice by injections of benzene (Lignac [37]) or indole (Büngeler [38]), and particularly in Büngeler's experiments, the relations between a severe alteration of the blood cells and the leukemia is striking. The five cases which I have selected from a large series of cases of acute leukemia indicate that at least in some instances leukemia is preceded by an excessive destruction of blood cells and that the patient may succumb to the anemia when the leukemic changes are still too insignificant to account for the lack of normal blood cells. In one case there were also evidences of a grave alteration of the granulated white blood cells. As a compensation for the excessive destruction of blood, a reactive myelopoiesis has been observed. Comparing the different foci of myelopoiesis, one obtains the impression that under the continuous stimulation of the destruction of the blood cells an increasing number of immature precursors of blood cells are called into existence until the hemocytoblastic stage is reached. Here and there, the hemocytoblasts may reveal attempts at maturation, but rapid multiplication seems to prevent their differentiation.

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33. Weil, P. E.: Presse méd. **33**:1297, 1925; Bull. et mém. Soc. méd. d. hôp. de Paris **48**:193, 1932.

34. Deloré and Bergomano: J. de méd. de Lyon **9**:227, 1928.

35. Falconer, E. H.: Am. J. M. Sc. **186**:353, 1933.

36. Vaughan, S. L.; Terplan, K., and Sanes, S.: Arch. Path. **18**:923, 1934.

37. Lignac, G. O. E.: Klin. Wchnschr. **12**:109, 1933.

38. Büngeler, W.: Klin. Wchnschr. **11**:1982, 1932; Frankfurt. Ztschr. f. Path. **44**:202, 1932.

It has been previously stated that several investigators have considered pathogenic relations between the aleukocytic conditions and the leukemia. I believe, however, that there are principal differences between the aleukocytic diseases and leukemia. Granted that in some cases of agranulocytosis, aplastic anemia and hemorrhagic aleukia the precursors of the blood cells may fail to mature because of the lack of a hypothetic maturation factor, the reverse of the formation of the blood cells to the hemocytoblastic stage occurs only in leukemia. It is also only in leukemia that throughout the body the mesenchyme acquires the potency to produce blood cells. In children particularly, agranulocytosis and aplastic anemia may lead to extramedullary myelopoiesis (Willi [39]), which, however, is entirely different from the leukemic changes.

As to the causative agent of the initial anemia, my cases do not yield any definite information. Three of the cases were in children and two were in adults. One of the adults was employed as dye mixer in a soap factory, but I have not been able to obtain exact information as to the nature of the dyes which he had used. Except perhaps in case 3, there were no relations to infections. To the trauma in case 1 I do not attribute any great significance. I believe that the child was already sick when the trauma was sustained and that the injury may perhaps have precipitated some of the symptoms. A traumatic etiology of leukemia is very doubtful, and what has been said about the relations between trauma and cancer (Ewing [40]) probably holds true also for leukemia.

SUMMARY

On the basis of a hematologic and histologic study of five cases of acute leukemia with very severe anemia which revealed a striking evidence of destruction of blood and a marked disproportion between the severity of the anemia and the extent of the leukemic changes, it is suggested that an initial abnormal destruction of blood cells may be of significance in the pathogenesis of acute leukemia.

39. Willi, H.: Jahrb. f. Kinderh. **142**:102, 1934.
40. Ewing, J.: Arch. Path. **19**:690, 1935.

Case Reports

DEFICIENCY OSTEOPOROSIS

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There is clinical evidence that deficiency osteoporosis is due to lack of vitamin D and hence may be an adult form of rickets.

The first cases were observed in Vienna in the autumn of 1918 [1] and in the cities of South Germany shortly afterward.[2] The ages of the patients varied from 15 to 80, while the sex distribution was the same as in the general population. The symptoms came on gradually and were of several months to one and a half years' duration. The patients complained of easy fatigability and pains in the ribs, sacrum and legs. The gait was waddling. Many patients showed deformity of the thorax and kyphoscoliosis of the thoracic spine. Bowing of the extremities was also present, particularly in older persons. Pelvic deformities were rare and were observed only in females. All of the bones were sensitive to pressure. Abductor spasms as well as positive Chvostek and Trousseau signs could be elicited in some cases. Tetany was fairly common. Edema of the lower extremities and anasarca were frequent. Most patients, especially the older ones, were markedly emaciated. Their diets consisted of potatoes, turnips, carrots, lean soups and war breads; proteins and animal fats were practically absent from their diets. Spontaneous fractures and infractions were described by Eisler [3] and Hass [4] as characteristic.

Partsch [5] described fifteen cases of deficiency osteoporosis pathologically, and his findings agree in general with those in this case. Looser [6]

1. Edelmann, A.: Wien. klin. Wchnschr. **32**:82, 1919; Wien. med. Wchnschr. **69**:800, 1919. Hahn, J.: Wien. klin. Wchnschr. **32**:713, 1919. Porges, O.: Wien. med. Wchnschr. **69**:801, 1919. Porges, O., and Wagner, R.: Wien. klin. Wchnschr. **32**:385, 1919. Zak, E.: Wien. med. Wchnschr. **69**:803, 1919.

2. Alwens, W.: München. med. Wchnschr. **66**:1071, 1919. Fromme, A.: Deutsche med. Wchnschr. **45**:510, 1919. Geisler, A.: Ueber das Krankheitsbild der "Hunger-Osteomalazie" bei Erwachsenen, Breslau, M. Bermann, 1919. Hamel, O.: Deutsche med. Wchnschr. **46**:68, 1920.

3. Eisler, F.: Wien. med. Wchnschr. **71**:482, 1919; Fortschr. a. d. Geb. d. Röntgenstrahlen **30**:67, 1923.

4. Hass, J.: Wien. klin. Wchnschr. **32**:677, 1919.

5. Partsch, F.: Deutsche med. Wchnschr. **45**:1130, 1919.

6. Looser, E.: Deutsche Ztschr. f. Chir. **152**:210, 1920; Verhandl. d. deutsch. path. Gesellsch. **2**:281, 1927.

claimed that late rickets and so-called juvenile osteomalacia are identical anatomically. In biopsies in his cases of rachitis tarda he found atrophy of the old bone, osteoid borders from 10 microns to more than 200 microns in width, a moderate number of osteoclasts and a fibrous marrow with a few small hemorrhages and lymphocytic foci. This picture, he claimed, is typical for rickets, and he regarded failure to calcify the osteoid as pathognomonic of rickets. The latter phenomenon was conspicuous in the case about to be described.

Miles and Feng [7] made chemical studies of the blood in ten cases of osteomalacia in women in northern and western China. Clinically, these cases were similar to those of deficiency osteoporosis. The usual diet consisted of a vegetable preserved in brine, wheat flour products or millet and practically no animal protein or fat. The blood calcium and phosphorus ranged from 5.2 to 7.4 mg. and from 1.8 to 3.8 mg. per hundred cubic centimeters of blood, respectively. When cod liver oil was added to the usual diet the patients showed a marked clinical improvement and a net gain in the blood calcium; tribasic calcium phosphate made the patients worse.

Dalyell and Chick [8] and Hume and Nirenstein [9] suspected that lack of some accessory food factor in the diets of their Viennese patients is the etiologic agent of deficiency osteoporosis. One hundred grams of cod liver oil administered over a period of one week cured the average patient. Phosphorus combined with oil was effective only if the oil was cod liver oil. Various vegetable oils and fats like rape and olive oils were ineffective. Patients ill more than a year gave a history of a summer remission and a winter relapse. It appears, then, that the accessory food factor lacking is vitamin D.

REPORT OF A CASE

A woman, aged 64, a domestic servant, had menstruated regularly from the twelfth to the fiftieth year; she had had nine pregnancies, eight deliveries and one abortion; she had always been undernourished and in poor economic circumstances. In July 1920 she gave a history of pain of two years' duration in both knee joints and in the lower part of the right leg. The right shoulder joint was slightly ankylosed. She received phosphorus with no improvement.

In August 1922 there were pains in the right hip joint, but shortening of the femur was not demonstrable. In October the right greater trochanter was 5 cm. above Nélaton's line with considerable shortening of the femur due to a lateral convex bowing in the region of the trochanters and the neck; the left greater trochanter was 1 cm. above Nélaton's line. Percussion and compression over the right trochanter were painful. There was a kyphosis of the thoracic spine.

Sun baths diminished the pain, but she had to use a cane. Symptoms became worse and a spontaneous fracture of the neck of the right femur occurred in

7. Miles, L. M., and Feng, C. T.: J. Exper. Med. **41**:137, 1925.
8. Dalyell, E. J., and Chick, H.: Lancet **2**:842, 1921.
9. Hume, E. M., and Nirenstein, E.: Lancet **2**:849, 1921.

January 1923. Phosphorus, 0.005 Gm. three times a day, was without effect. In June 1924 the patient began to show evidences of cardiac decompensation and died the following August.

The diagnosis was osteoporosis, atrophic fracture of the neck of the right femur, heart failure and edema.

The autopsy showed severe anasarca. The right lower extremity was shortened and rotated strongly inward, and there was a right-angled bend in the femoral

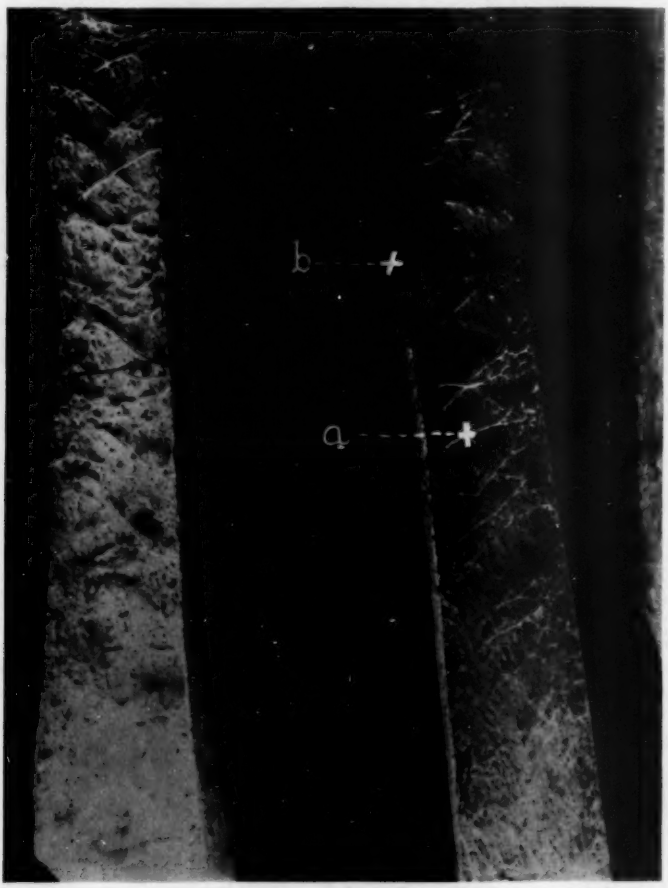

Fig. 1.—Midshaft of the right femur, showing an extreme degree of porosis: (*a*) disappearance of the spongiosa except for fine trabeculae, with resulting widening of the marrow cavity; (*b*) atrophy of the cortex.

shaft. Above the condyles, 5 cm. from the joint surface, there was a recent complete slightly impacted fracture with a large circular subperiosteal osteophyte. The marrow cavity of the midshaft region was 3.4 cm. wide and contained hyperemic fatty marrow. The cortex in this region was about 0.5 cm. in maximum thickness and was made up of one or two separate paper-thin bony trabeculae. The spongiosa was sparse here (fig. 1). The kinked portion of the shaft, how-

ever, had a marrow cavity 1 cm. wide, and the cortex on the convex side of the kink was 1.9 cm. thick, this thickening having resulted from a callus with the structure of a soft, compressible, uncalcified spongiosa. The spongiosa at either end of the femur was extremely porotic.

The right tibia showed a subperiosteal fracture 3.5 cm. above the ankle joint with widespread hemorrhage into the marrow.

The cartilages of the right knee and ankle joints in places showed almost complete defects which extended practically to the underlying bone. The right patellar cartilage had almost entirely disappeared.

The left femur was normal in shape. The cortex varied from 0.1 to 0.3 cm. in thickness; its structure was that of a spongiosa consisting of thin parallel longitudinal trabeculae. The spongiosa of the condyles and head was very porotic. The marrow cavity at the midshaft was 2.1 cm. wide.

The left tibia was not altered in shape. Its cortex was 0.2 cm. thick and its marrow cavity markedly widened.

The pelvis was distorted, the symphysis pubis being pushed to the left. The lumbar spine was moderately bowed with a right scoliosis. The upper thoracic spine showed a strong arcuate kyphosis. The lateral diameter of the thorax was much reduced.

The skull was mesocephalic; the maximum thickness was about 0.6 cm. Neither the external nor the internal tables were recognizable. The calvarium also had the structure of a soft porotic spongiosa.

The upper extremities showed no deformities.

All the bones could be easily cut with the knife.

Microscopic Observations.—The right femur, the right patella and the vault of the skull were examined microscopically.

The periosteum was of variable but normal thickness and, in the deeper layers of the cambium, showed lymphocytes and erythrocytes, solitary or in groups, as well as intracellular and extracellular blood pigment.

The extraordinarily severe generalized diminution of osseous tissue of the skeleton was especially marked in the lower two thirds of the femur (fig. 2) and in the patella. Only exceptionally was it 2 and rarely 3 trabeculae thick. The cortex of the neck and subtrochanteric region of the femur was of normal thickness or even thicker than normal, this difference apparently representing a compensatory change to meet the mechanical stresses on the femur in this region (fig. 3*A*). But even so the cortex here had the structure of a wide-meshed spongiosa formed by a few thin irregularly distributed trabeculae, which were most numerous beneath the periosteum. The marrow spaces between the trabeculae were filled chiefly with fibrous marrow, apparently representing additional efforts on the part of the body to meet the mechanical stresses in this region.

The high grade osteoporosis manifested itself also in the enormous number of lacunar resorption spaces found everywhere. The periosteal surface of the femur for long stretches was eaten away. Cortical and spongiosa trabeculae in all regions were extremely thin because of this lacunar resorption (fig. 3*B*). Long and short interruptions (fig. 2) in the cortex of the long bones were further evidence of this most severe bone atrophy. In contrast to Paget's disease and the degree of destruction here, osteoclasts were relatively rare.

Osteoid played a most important part in the skeletal structure, but its distribution was irregular (fig. 3*B*). There were places where the osseous tissue consisted entirely or almost entirely of osteoid, other places where it was formed equally of osteoid and calcified bone, and still other places where, though the osteoid was comparatively diminished, nevertheless it was pathologically increased.

The points of greatest mechanical stress were best calcified—i. e., the outermost portions of the tubular bones—in agreement with the calcification law of Erdheim. The lower two thirds of the femoral shaft (fig. 2) and the cortex of the anterior portion of the patella showed the least osteoid and the most calcified bone. Much osteoid was found in the trabeculae of the spongiosa-like cortex of the crooked portion of the femur, in lacunar resorption spaces in general and in haversian canals. There was little osteoid in the spongiosa because the spongiosa had practically disappeared.

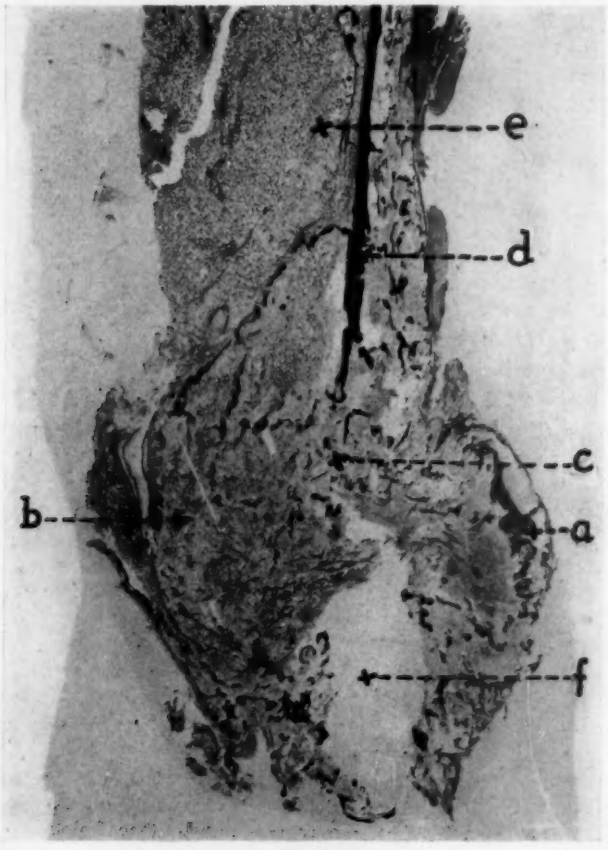

Fig. 2.—Subperiosteal fracture of the femoral shaft; × about 30: (*a*) periosteal callus; (*b*) endosteal callus; (*c*) intermediate callus; (*d*) extremely thin but well calcified cortex with interruptions in the cortical continuity; (*e*) disappearance of the spongiosa; (*f*) artefact—a pulling out of the lower end of the fracture fragment on sectioning.

The thickness of the osteoid varied greatly. Normally osteoid does not exceed 10 microns in thickness, according to Pommer.[10] Two thousand, seven hundred

10. Pommer, G.: Zentralbl. f. Herz.- u. Gefässkr. **16**:342, 1924; Arch. f. klin. Chir. **136**:1, 1925.

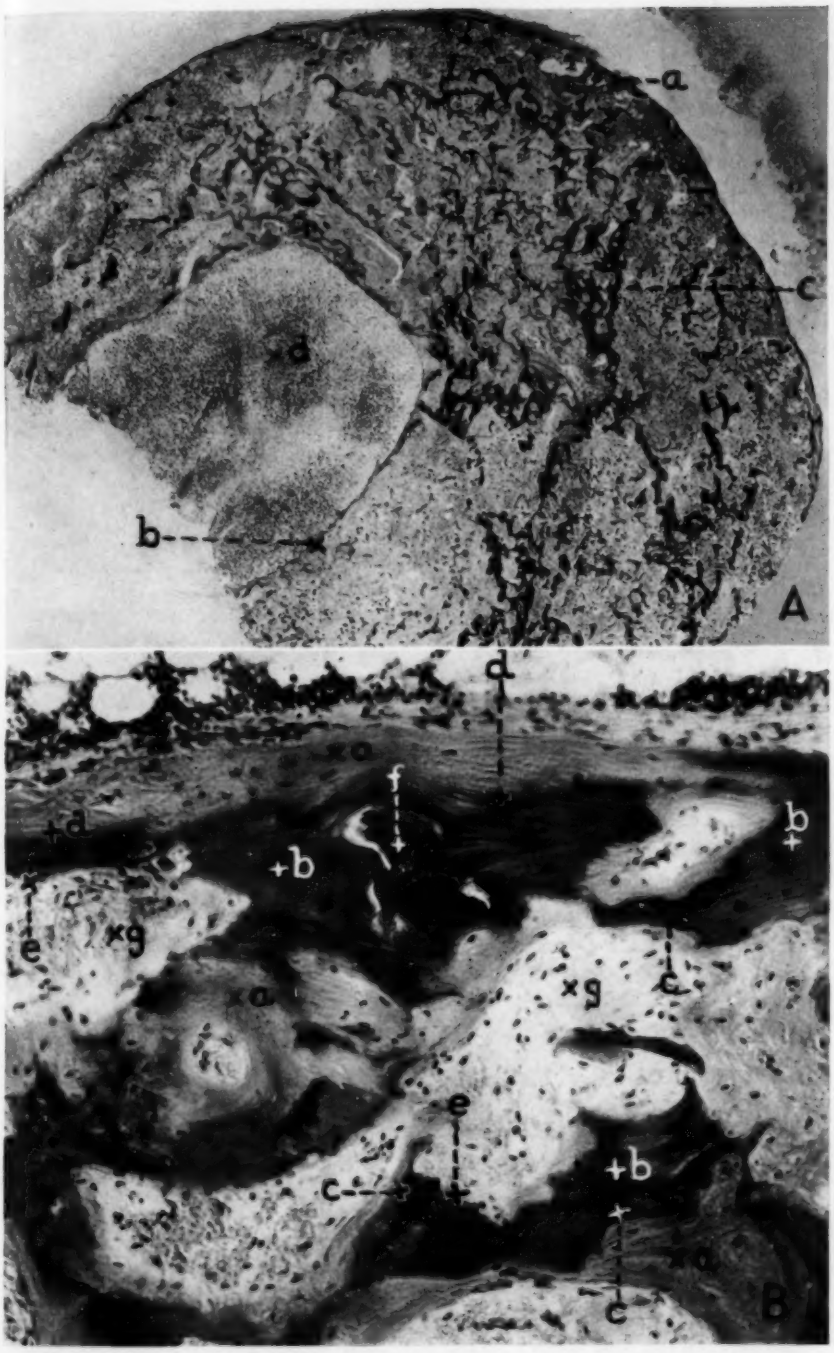

Fig. 3.—*A*, transverse section of the subtrochanteric region of the right femur; × about 30: (*a*) external boundary sheath; (*b*) internal boundary sheath; (*c*) trabeculae spanning the space between the external and internal boundary sheaths and forming a thick wide-meshed porotic spongiosa at the site of the cortex; (*d*) marrow cavity.

B, section from the subtrochanteric region; × 100: (*a*) osteoid, lamellated in structure; (*b*) calcified bone; (*c*) cement line; (*d*) appositional resting line; (*e*) lacunar resorption of calcified bone; (*f*) micro-fracture, Y-shaped, with fibrinoid in the fracture space; (*g*) fibrous marrow between the trabeculae.

and seventeen (2,717) measurements were made. The average values varied in different regions. In the femur, osteoid was thickest in the crooked portion and thinnest in the lower two thirds of the shaft. The average thickness was greater in the patella than in the femoral condyles and least in the calvarium. The minimum measurements in all regions examined were within normal limits.

The osteoid structure was not uniform. In some places it had a distinctly lamellated structure with few osteoblasts. In other places it was fibrillated, plexiform and rich in osteoblasts. And then, again, in many places it appeared to have a homogeneous structure. The boundary between mature and immature bone was a lacunar cement line, an appositional resting line or a wavy granular zone of Pommer (fig. 3*B*). Osteoid was not always present as a border or margin on the surface of calcified bone. Many times the deeper or central parts of the osseous tissue remained uncalcified, while near the free surface there was a discontinuous calcification, a phenomenon often present in rickets. Partial calcification of osteoid was common. This sometimes took the form of small isolated dark blue granules of calcium within the osteoid; sometimes the process was homogeneous, the osteoid staining a pale blue indicating incomplete calcification.

Measurement of the Thickness of the Osteoid Tissue

Region	Measurements	Thickness, Microns		
		Average	Maximum	Minimum
Femoral head	41	24	80	8
Trochanteric region	459	49	272	4
Bowed portion of the upper part of femoral shaft	1,129	58	512	4
Straight portion of the lower part of femoral shaft	468	24	96	4
Femoral condyles	269	25	128	4
Patella	209	34	352	4
Calvarium	142	20	51	4
Total measurements	2,717			

The chondro-osseous junctions showed characteristic lesions. The old calcification zone and the old osseous junctional plate had been largely destroyed from below, the destructive process often extending into and beyond the transitional zone of the joint cartilage (fig. 4). Later a new preparatory calcification zone might be formed on the new internal surface of the cartilage, but this new calcification zone was markedly advanced toward the free joint surface. In the most favorable instances the new preparatory calcification zone was covered by a new, though thin, poorly calcified, irregular, discontinuous osseous junctional plate. Yet even the new preparatory calcification zone might be absent so that the new osseous junctional plate and the uncalcified cartilage were in direct contact. The trabeculae of the underlying spongiosa inserted in the osseous junctional plate if one was present; in the absence of one, they inserted in the preparatory calcification zone; and if the latter was also absent, the trabeculae rested directly on uncalcified cartilage. At the edge of the joint the cortex joined the osseous junctional plate as is normal or the joint cartilage ended abruptly in a calcification zone into which the cortex inserted.

Destruction of the original joint cartilages examined (right femoral head, condyles and patella) was severe. In the patella, for example, there were only small rests of old cartilage. Rossi [11] and Rabson [12] have shown that disuse, of

11. Rossi, A. L.: Virchows Arch. f. path. Anat. **284**:256, 1932.
12. Rabson, S. M.: Virchows Arch. f. path. Anat. **291**:624, 1933.

which ankylosis is the most complete form, causes marked destruction of the joint cartilage proper. This patient was bed-ridden over a period of years. The cartilage showed lacunar resorption, but it also showed incomplete destruction as described by Pommer, this change being preceded by the formation of Weichselbaum's spaces. The normal basophilic staining of the cartilage had been largely replaced by a red-violet stain, further evidence of damage. Pads of new cartilage (fig. 4) were present in the indentations eaten out of the internal surface of the old joint cartilage or between the old cartilage and the new osseous junctional plate.

Fractures and infractions have been described, in regard to roentgen appearance,[13] as characteristic of deficiency osteoporosis. There was a typical subperiosteal fracture without displacement in the lower portion of the femur

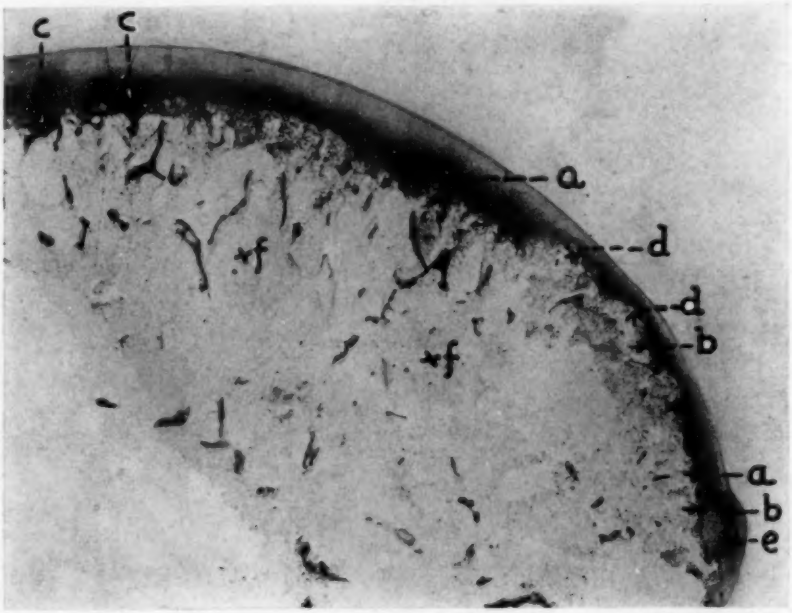

Fig. 4.—Section of the femoral head (× about 30) showing the joint cartilage and subjacent spongiosa: (*a*) disappearance of the old calcification zone and osseous junctional plate; (*b*) new calcification zone and new osseous junctional plate; (*c*) pads of new cartilage between the new osseous junctional plate and the old cartilage; (*d*) deep indentations eaten out of the cartilage from below, extending up to and into the transitional zone; (*e*) hyperplasia of the old cartilage; (*f*) extreme porosity of the spongiosa of the femoral head, showing the short, thin, widely spaced and scanty trabeculae.

(fig. 2). The periosteum and the bone marrow in the vicinity of the fracture were neither torn nor interrupted. The callus was ring-shaped with the upper and lower fragments inserted into it. It had three divisions—periosteal, endosteal

13. Kienbock, R.: Fortschr. a. d. Geb. d. Röntgenstrahlen **33**:862, 1925. Milkman, L. A.: Am. J. Roentgenol. **24**:29, 1930. Eisler.[3]

and intermediate. Periosteally and endosteally it was bounded by a thin partially calcified shell of bone, within which there were a few thin, irregular trabeculae, poorly calcified and in places cartilaginous, and fibroblasts, disseminated fat and marrow cells, leukocytes, blood pigment and a moderate number of necrotic areas.

Microfractures were also present (fig. 3*B*). These showed more or less gaping fracture spaces, frequently Y-shaped, containing reddish fibrinoid, a sign that they were not artefacts. Doubtless the crook-shaped bend of the subtrochanteric region of the femur was the result of repeated microfractures which had healed. Small compact calluses consisting of a mass of primitive osteoid with isolated calcium deposits and mature hyaline cartilage were present in the bent

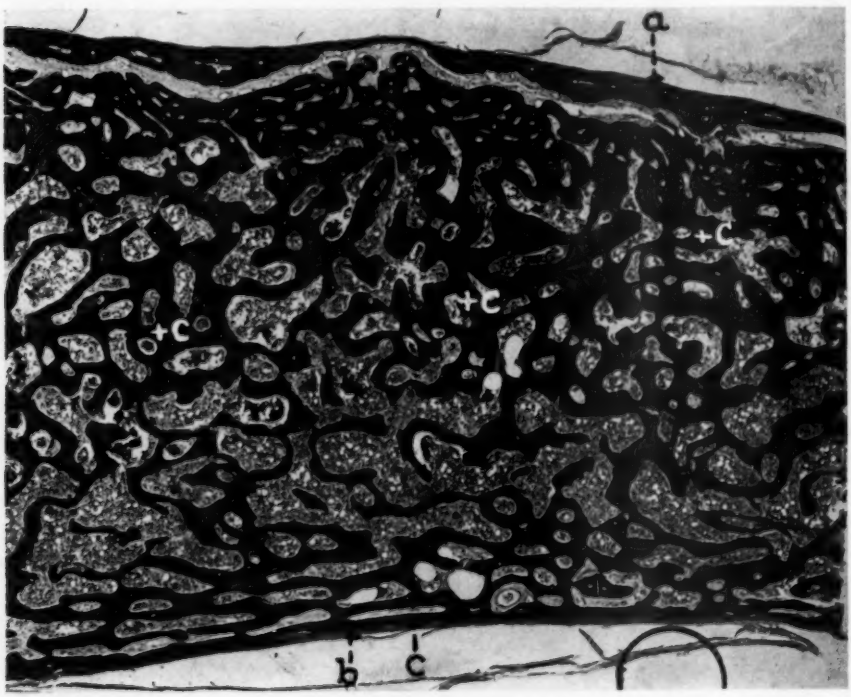

Fig. 5.—Section of the calvarium; × about 30: (*a*) pericranial portion showing the disappearance of the external table and its replacement by a thin sheath of bone; (*b*) dural portion showing the replacement of the internal table by a thin sheath of bone; (*c*) trabeculae forming a spongiosa replacing the normal diploic structure.

portion of the femur and in the patella and were to be interpreted as the healing stages of microfractures.

The tables of the calvarium with the intermediate diploic structure, as was to be expected, were gone (fig. 5), and the calvarium consisted of a spongiosa bounded durally and pericranially by thin sheaths of bone. At death, in spite of this remodeling, lacunar resorption was only moderate. The calcium content of the calvarium was much nearer normal than that of the femur, and osteoid was present

only in moderate amount (table). All in all, however, in comparison with the normal this calvarium was obviously atrophic.

Unfortunately the parathyroids were mislaid and hence were not available for microscopic study.

SUMMARY

A case of deficiency osteoporosis is described in a 64 year old white woman. Clinically, deficiency osteoporosis appears to be due to lack of vitamin D. The changes at the chondro-osseous junctions in deficiency osteoporosis are similar to those in rickets. Osteoid is markedly increased in amount and thickness as it is also in rickets. The last two changes are considered as pathognomonic of rickets. Hence there is some histologic evidence that deficiency osteoporosis and rickets may be the same entity.

RETICULO-ENDOTHELIAL SARCOMA OF THE SPLEEN

REPORT OF A CASE

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AND
META NEUMANN, M.S.
WASHINGTON, D. C.

About 120 cases of primary sarcoma of the spleen have so far been reported in the literature. The case described in this article is the only one in 6,400 autopsies performed at this hospital since 1884. There have been 7 cases of metastatic carcinoma in this series.

Smith and Rusk,[1] in 1923, made a survey of primary malignant tumors of the spleen, classifying the 104 cases which had been reported up to that time. They found a lack of uniformity in the terminology. As they brought out, there are three types of tissue in the spleen from which neoplasms may arise:

> . . . (1) the capsular and trabecular framework which may give origin to fibroma and fibrosarcoma; (2) the lymphoid elements from which may arise either a simple lymphoma (at times so-called lymphadenoma) or a malignant lymphoblastoma, that is, the so-called lymphosarcoma, and (3) angiomas and cavernous angiomas, together with the malignant counterpart, the endothelioma, arising from the vascular or sinus endothelium.

The tumors usually grow rapidly, and the diagnosis is usually made at necropsy.

Smith and Rusk decided that lymphosarcoma is probably the most usual type, although endothelioma is not uncommon. They reported 2 cases in which a tumor arose from the lining cells of the splenic sinuses. The cells assumed a definite alveolar arrangement for the most part. In some areas they were closely packed together with only fine connective tissue fibrils between them, as shown by Mallory's connective tissue stain. The cells had a fairly definite outline, spindle-shaped or polygonal, with clear, oval or rounded nuclei, the smallest being twice the diameter of lymphoid cells. Of the cases reported since that survey, we review briefly only those in which the microscopic picture was somewhat similar.

From the Blackburn Laboratory, Saint Elizabeths Hospital.

1. Smith, C. E., and Rusk, G. V.: Arch. Surg. **7**:371, 1923.

Taylor [2] described a spleen with a few atrophic malpighian bodies and an increase in the endothelial structures. The blood channels were large, poorly formed and lined by cells which were larger and more deeply stained than those normally present. There were many hyperchromatic cells with several closely packed nuclei or with one large vesicular nucleus. Mitotic figures were numerous. Taylor designated the endothelium of the splenic pulp as the origin of this tumor.

In Howard's [3] case the clinical picture was that of a pernicious anemia. The spleen weighed 1,800 Gm., and there was metastasis only to the liver. The tumor cell was a reticulum cell with a round or ovoid vesicular nucleus and fairly abundant cytoplasm. There were occasional giant cells and many mitotic figures. Among these cells were varying numbers of lymphocytes, and a delicate reticulum was present throughout.

Wright and Stevenson [4] described a sarcoma of the spleen composed of large cells and showing an alveolar arrangement. The main constituent of the tumor was a round cell with a fairly large nucleus, probably of mesoblastic origin. Cells of that type filled the meshes of a fine reticular stroma and produced a somewhat alveolar structure.

Paine [5] reported another malignant neoplasm of the spleen which he called hemendothelioma. The spleen was enlarged and nodular. Some of the nodules were white and firm and were necrosed in the center. The nodules were separated by coarse and fine strands of fibrous tissue which from the arrangement beneath the capsule appeared to grow from the original splenic trabeculae. The normal pattern of the spleen was masked. Macroscopically, no malpighian corpuscles were identified. There were metastases to the liver and the bone marrow. The cells varied from a spindle-shaped form with a deeply stained nucleus to a nearly circular form with a vesicular nucleus. They resembled endothelial cells. Some of the cells showed phagocytic properties, and their tendency to form tubules indicated their endothelial origin. Intracellular fibrils were not demonstrated.

Caldwell [6] summarized the observations in his case as follows: There was a primary malignant neoplasm with extensive metastases to the lymph nodes, lungs and subcutaneous tissues, together with numerous peritoneal implantations. The tumor was of the large round cell type with a tendency toward alveolar arrangement but without any definite angioplastic structure. Delicate argyrophilic fibrils seemed to

2. Taylor, A. L.: Bristol Med.-Chir. J. **46**:121, 1929.
3. Howard, T.: J. Lab. & Clin. Med. **14**:1157, 1929.
4. Wright, T. H., and Stevenson, E. M. K.: Glasgow M. J. **114**:1, 1930.
5. Paine, C. G.: J. Path. & Bact. **34**:139, 1931.
6. Caldwell, G. T.: South. M. J. **26**:120, 1933.

be formed by the tumor cells in the more differentiated portions of the tumor, but they were nearly completely lacking in the more cellular areas. The tumor was considered to be a primary reticulo-endothelioma of the spleen.

REPORT OF CASE

A well developed and well nourished white man of 42 years was admitted to the hospital on July 15, 1930, suffering from dementia paralytica. Previous to his admission he had received treatment with arsphenamine, mercury and malaria. The malaria terminated spontaneously after twelve chills. Serologic and neurologic findings confirmed the diagnosis of dementia paralytica. The patient showed complete mutism.

The clinical course was uneventful for nearly three years; then the inguinal lymph nodes began to enlarge. Abdominal palpation showed a large, hard, nodular mass at the crest of the left ilium. The roentgenologist's report read: "There is a disintegration of the wing of the left ilium. There is no evidence of a bony growth such as one would expect if the process were carcinomatous. It is possible that the condition is sarcoma." Further studies revealed numerous metastatic growths, but the location of the primary lesion could not be ascertained.

A biopsy specimen of an inguinal lymph node was reported on as follows: "The lymphoid tissue is practically completely destroyed and infiltrated by a new growth consisting of rather large polyhedral and spindle-shaped cells with large vesicular nuclei. There is a moderate amount of connective tissue stroma, rather slight on the whole, and small immature blood vessels. Occasional giant cell formation is observed. Many of the nuclei are large, undergoing mitotic division. The cells on the whole appear more of epithelial type although rather undifferentiated. In addition to the main cells there are numerous small rounded bodies, often slightly oval, staining intensely by hematoxylin and bearing no definite relationship to the cell, although they are occasionally found in the cell and even in the nucleus. They do not seem to indicate degeneration because there is not much necrosis present." The diagnosis was metastatic carcinoma.

Histologic examination of the tumor of the bone showed the following characteristics: "The appearance of the growth varies greatly from place to place. A section taken through the soft tissues shows an invasive growth made up of cells irregular in size and shape, with very little stroma. The cells have large nuclei and a tendency toward a fusiform or rounded cytoplasm. Nucleoli are present in some of the nuclei, and occasionally multiple nuclei are found in the same cell. There is invasion of the muscular tissue adjacent to the bone, infiltration between the separate fasciculi and degeneration of the muscular tissue. One of the sections from the bone shows rather normal-appearing red marrow; another shows a markedly increased number of small rounded oval cells and scattered large cells, considerably resembling those of the adjacent growth." The pathologist offered the following comments: "In comparison with the lymph node previously submitted, the tumor is apparently of the same nature, and is probably of epithelial origin. From the undifferentiated character of the cells, I should be inclined to regard it as highly malignant. I should hesitate to suggest the point of origin of this tumor, but the cells resemble those which might be derived from the lung or from the kidney." The histologic diagnosis was metastatic carcinoma.

At the time of this biopsy the patient's blood showed 74 per cent hemoglobin and an erythrocyte count of 4,010,000 cells per cubic millimeter. Two weeks later the hemoglobin content dropped to 45 per cent and the number of red blood cor-

puscles to 2,430,000. One month later the hemoglobin content was 44 per cent, and the red cells numbered 2,310,000. The white blood cell count remained normal throughout. The blood chemistry and electrocardiograms were not unusual. The basal metabolic rate was plus 20.2.

Considerable edema of the lower extremities appeared in the later stages. The patient showed progressive mental and physical deterioration. He died four months after the appearance of the first sign of neoplastic disease.

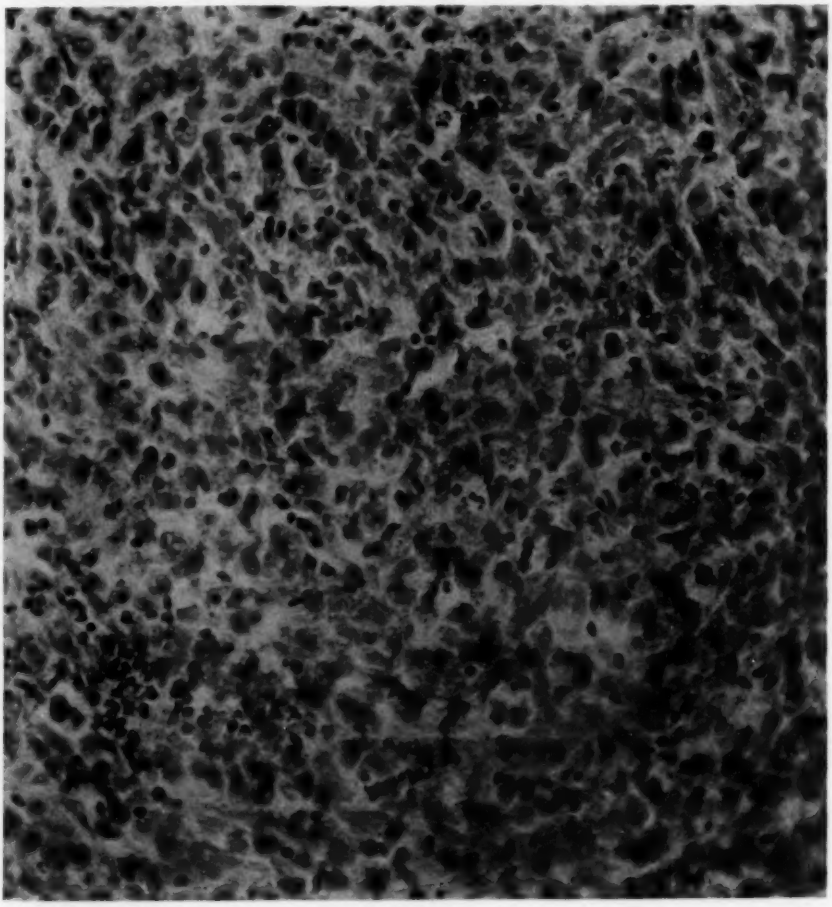

Fig. 1.—Section of the lymph node removed for biopsy. Hematoxylin and eosin stain; high power magnification.

A summary of the autopsy observations (made by Dr. Walter Freeman) follows: The body was poorly nourished. There was a large node in the left axilla. Two ribs showed tumor formation and pathologic fracture. There was induration of the left iliac bone. The parietal and visceral peritoneum showed numerous flat grayish plaques and was considerably thickened. The liver was

enlarged. Scattered areas of metastasis were found in the epicardium. Small tumor nodules were found in both lungs. These tumors were embedded in the tissue rather than compressing it. There were several indurated ulcers in the ileum. Metastatic growths were found in the serosa of the appendix and in the cecum, and a large nodule lay between the bladder and the rectum. The liver contained only one nodule, 2 cm. in diameter. There was surprising preservation of the hepatic architecture in this nodule, and no obvious compression of the surrounding hepatic

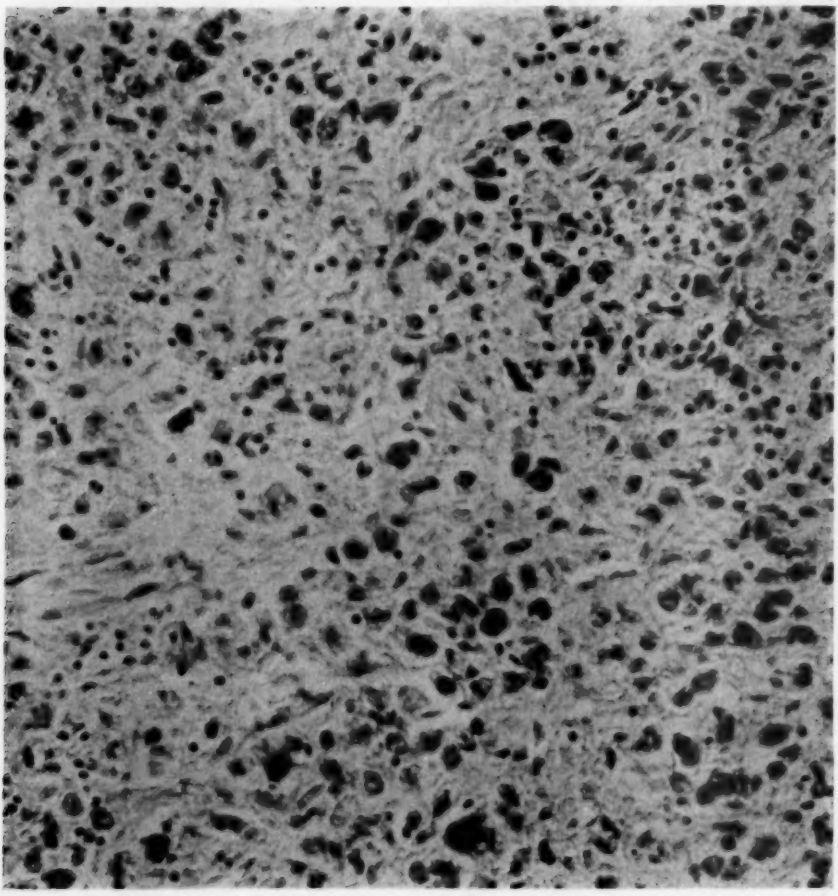

Fig. 2.—Reticulo-endothelioma of the spleen. Hematoxylin and eosin stain; high power magnification.

tissue, as would be usual in the ordinary metastatic carcinoma. Metastatic nodules showing similar characteristics were also found in the pancreas.

The spleen weighed 695 Gm. It was dark, fairly firm and nodular. On incision it was divided up into a number of nodules, the largest being about 5 cm. in diameter. Most of these were dark red, showing much of the color of

splenic tissue, except that in the center of some of them there was a stellate area of whitish color, which was slightly firmer than the surrounding dark splenic-appearing tissue but showed no tendency to necrosis. There was a little normal splenic tissue between the various lobules.

Confluent enlarged nodes were found along the abdominal aorta, around the left kidney, along the iliac and pancreatic vessels and in the left axilla. There

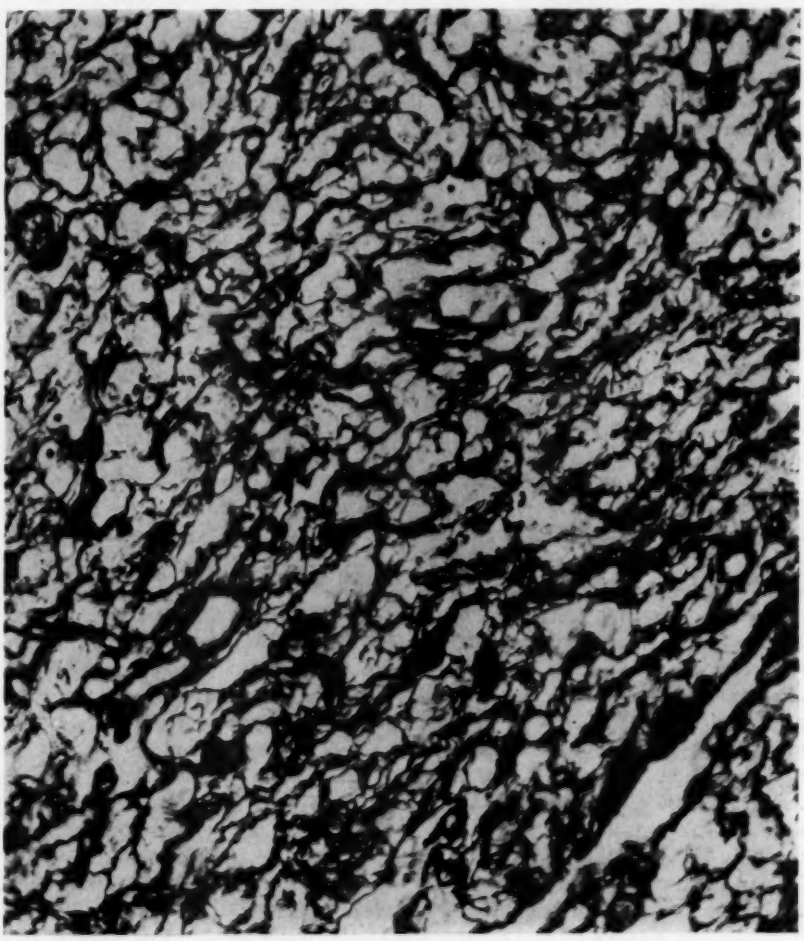

Fig. 3.—Reticulo-endothelioma of the spleen, showing argyrophilic reticulum. Perdrau stain; high power magnification.

were several nodules in the substance of each kidney. The bladder showed evidence of metastasis. The prostate and testes were normal. The endocrine system was normal, except for the right adrenal gland, in which metastasis was noted.

There was practically complete destruction of the sixth and seventh ribs in the axillary portion by the tumor involvement. A growth protruded from the

eighth and ninth ribs into the thorax and was adherent to the lung. A large mass appeared in the left ileum, which projected into the pelvis and disrupted the bone. There was also rather massive infiltration of the lumbar vertebrae. There were a few swellings beneath the pericranium which did not appear to invade the bone.

The brain apparently was normal.

The diagnosis at the time of autopsy was that there were possible sources of primary growth around the pelvis of the left kidney or the pancreas, with metastasis to the epicardium, lung, spleen, lymph nodes, peritoneum, liver, bladder, adrenal glands, ribs, vertebrae and ilium. However, it was mentioned that "the only other growth having definite appearances of primary carcinoma is in the spleen, where the foci are multiple and the character of the tissue entirely different from that seen in the other organs. The extent of the carcinomatous invasion of the bony system and of the peritoneal surface is quite unusual in this case, but the very great involvement here and the slight involvement in the thoracic cavity are a cause for comment."

Microscopic examination shortly after autopsy confirmed the first impression that the cell type was carcinoma originating in the region of the left kidney. However, it was mentioned that "the metastases are quite generally characterized by their infiltrative tendency, thus reminding one of lymphoid growths rather than carcinoma."

Detailed microscopic examination gave the following results: One section of the spleen included a portion of the tumor nodule with the whitish center, as described at autopsy, and also some compressed adjacent splenic tissue. The pallor of the center was due to the absence of blood and the scarcity of blood spaces in that area. There was a fairly definite fibrous capsule separating the neoplasm from the adjacent splenic pulp. This strip was not continuous and did not act as a barrier, as tumor cells were seen in the wall of a vessel which lies in this strip of connective tissue, also scattered in the pulp on the other side. A few trabeculae were seen in the sarcomatous area near the capsule.

In another section of this organ no trace of normal architecture was preserved. Except for the capsule, the splenic pulp had been entirely replaced with undifferentiated neoplastic cells. These cells were large, often attaining a size of 30 microns or more, with single or multiple vesicular nuclei and one or two prominent nucleoli. The rim of clear cytoplasm was usually narrow and assumed various shapes. Some of the cells were spindle-shaped with a long narrow nucleus; others were round, oval or polyhedral. Giant cells were fairly numerous. Mitotic figures were frequently seen. Small dark blue granules were often gathered in small groups enclosed in a cell body, but without nuclear membrane. Since the cells in which they appeared did not possess other visible nuclei, it was conjectured that they represented nuclear material or fragments of chromatin due to karyorrhexis. Sometimes similar-appearing granules were free in the tissue.

The few remaining malpighian corpuscles were small. Lymphocytes were scattered throughout the section, as well as a moderate number of polymorphonuclear leukocytes. Trabeculae were not recognized. The capsule was intact. Red blood cells were seen everywhere, but the walls of the vessels were thin and indistinct.

With the Perdrau stain there was seen to be an extremely dense anastomosing stroma of argyrophilic fibers everywhere. The vessels were now easily identified. The contrast with a normal spleen stained by this method was striking. In the normal section, connective tissue fibers were present only in the trabeculae and walls of sinuses. With Mallory's aniline blue connective tissue stain the picture

was about equally striking. Almost every individual cell appeared to be surrounded by a network of delicate and coarse fibrous strands.

The tumor cells were entirely similar in every metastasis. Studies were made of the metastases which occurred in the epicardium, lungs, kidneys, liver, pancreas, intestine, adrenal glands, omentum and ileum. A peculiar characteristic of this case is the intermingling of tumor cells and parenchyma without compression of the latter. The individual structure of the organ could be discerned underlying the neoplasm, except in the spleen, where the characteristic structure had been completely lost in the tumor. From our study of the case we concluded that the primary focus of the growth could not have been the kidney or the pancreas. From our microscopic study it appears that the "stellate areas of whitish color" described at autopsy are centers of neoplastic growth. There is no doubt from the type of cells and their arrangement that we are dealing with a sarcoma instead of a carcinoma. The formation of an argyrophilic reticulum is proof that we are dealing with a mesodermal growth.

We classify it, therefore, as a primary sarcoma of the spleen originating in the reticulo-endothelial cells.

COMMENT

The lack of uniformity in the classification of splenic tumors has repeatedly been stressed. Ewing[7] divided them into three groups: spindle cell sarcoma, endothelial sarcoma and lymphosarcoma. He expressed the belief that those arising in the reticulo-endothelial system are of most frequent occurrence. His description follows:

> As a rule, it produces multiple nodules in a greatly enlarged organ, and many of these nodules may fuse into larger diffuse masses. Metastases are commonly present. . . . The tumor is, therefore, quite malignant.
>
> The structure consists of large cells with single or multiple vesicular nuclei and pale cytoplasm. They are round or elongated or polyhedral, and giant-cells may form. The arrangement may be diffuse or alveolar.

Ewing did not mention the argyrophilic reticulum, which is a constant feature of tumors of reticulo-endothelial origin. The details of our observation show a great similarity to Ewing's description of endothelial sarcoma. We suggest, however, that all neoplasms of this nature be definitely classified as reticulo-endothelial sarcoma. This will avoid confusion with the angioplastic tumors arising in the endothelial lining of the sinuses.

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7. Ewing, T.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1931, p. 422.

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7. Ewing, T.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1931, p. 422.

GENERALIZED PRIMARY ANGIOSARCOMATOSIS OF THE LYMPH NODES

PERRY J. MELNICK, M.D.
DECATUR, ILL.

The following report concerns a unique case of generalized primary angiosarcomatosis of the lymph nodes. Only two cases resembling this one have been reported. Baumgarten [1] in 1916 described a case of generalized primary spindle cell sarcoma of the lymph nodes. Kaufmann [2] mentions a case of generalized primary myxosarcoma of the lymph nodes which he observed. A doubtful case reported by Spieler [3] seems to be that of a reticulum cell lymphosarcoma (retothel sarcoma) of the lymph nodes and intestine.

The familiar lymphosarcomas are by far the most frequent of the primary tumors of the lymph nodes. The so-called endotheliomas of lymph nodes are undoubtedly retothel sarcomas, their histogenesis being from the sinus endothelium. Other primary sarcomas of the lymph nodes are rare. Kaufmann mentioned cases of fibrosarcoma, spindle cell sarcoma, angiosarcoma and round cell sarcoma. In these cases one lymph node or one group of lymph nodes was involved.

Our case is very curious and unique in that almost every lymph node in the entire body appears to be the site of apparently primary angiosarcoma. Baumgarten's case was that of a 58 year old man in whom at autopsy all the lymph nodes in the entire body were enlarged up to the size of hen's eggs and were the site of primary spindle cell sarcoma. No trace of tumor was found anywhere else. Kaufmann's case was of a 32 year old man with generalized lymph node enlargement of fifteen years' duration, in whom the lymph nodes were the site of myxosarcoma. A case ascribed by Spieler to this group turns out to be one of reticulum cell lymphosarcoma (retothel sarcoma) of the lymph nodes and lymphatic tissue of the intestine. In his detailed description of the case the reticulum cells are fully identified even to the intracellular argentophil reticulum fibrils; but at the time of his report (1918) retothel sarcoma had not yet been clearly recognized as one of the three forms of lymphosarcoma.

From the Department of Pathology, Cook County Hospital; Dr. R. H. Jaffé, director.

1. Baumgarten, P.: Berl. klin. Wchnschr. **52**:1201, 1915.

2. Kaufmann, E.: Pathology for Students and Practitioners, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 282.

3. Spieler, F.: Zur Lehre des generalisierten Sarcoms der Lymphdrüsen und des Darmes, Inaug. Dissert., Basel, 1918.

REPORT OF A CASE

The patient, a 64 year old Italian laborer, is alive and well. He entered the Cook County Hospital on Oct. 6, 1933. About a year previously he first noticed lumps in the neck, both axillary regions and both inguinal regions. The lumps grew slowly and never caused him any trouble. Four months before entrance he became short of breath and began to have swelling of the ankles. He had rheumatism twenty-four years before entrance. There was nothing else of significance in the past history, and the family history was negative.

He appeared well developed and well nourished, comfortable and not acutely ill. The pulse, temperature and respirations were normal. The blood pressure was 100 systolic and 55 diastolic. Examination of the head showed no abnormality. In the neck every group of lymph nodes on both sides was enlarged. The size of the nodes ranged to that of a cherry, and they were firm, discrete and movable. In the chest crackling râles were heard in both lower lobes, but there were no other abnormal findings. The heart, on percussion, appeared slightly enlarged to the left; a systolic murmur was heard over the apex, transmitted to the axilla. In both axillary regions the lymph nodes were enlarged to walnut size. They were discrete and firm. In addition, every other group of lymph nodes in this region was enlarged, namely, subpectoral and interpectoral nodes. These could not always be seen but could be felt as pea-sized movable nodes. The abdomen was soft and not tender. The liver was palpable 2 fingerbreadths below the costal margin. The spleen and kidneys were not palpable; no abnormal masses were felt. In the inguinal regions the lymph nodes were enlarged to walnut size, discrete and firm. In the upper extremities the epitrochlear lymph nodes were enlarged to cherry size, and several smaller nodes were palpable, forming a chain along the medial aspects of both arms. The lower extremities had slight pitting edema about the ankles.

Leukemia, lymphosarcoma or Hodgkin's disease were the possibilities that came to mind. The cardiac findings were interpreted as a rheumatic mitral lesion with myocardial decompensation. Examination of blood films revealed no abnormal cells. There was a moderate anemia; the hemoglobin content was 66 per cent; the red blood cell count, 3,470,000; the white blood cell count, 10,200. Stained films revealed no abnormal blood cells. The urine was normal; the blood chemistry was normal and the Kahn reaction of the blood negative. An x-ray film of the chest revealed only slight increase in the hilus markings. X-ray films of the gastrointestinal tract and of the skeleton revealed nothing abnormal.

A biopsy of a cervical lymph node disclosed what was apparently an angiosarcoma associated with a lymph node. A biopsy of another cervical lymph node revealed an exactly similar picture. This unusual finding stimulated much interest in the case. Still another biopsy was made, and this time two large cherry-sized lymph nodes were carefully dissected out from the right epitrochlear region and immediately fixed in Zenker's fluid containing dilute formaldehyde solution. The patient made an uneventful recovery from the biopsies and with digitalis therapy for his heart condition improved so markedly that he insisted on going home. He is at the time of writing alive and well, thirty months after the first appearance of the enlargement of the lymph nodes. A recent examination revealed nothing additional of importance. The nodes have increased only very slightly in size in the past fifteen months, and are giving the patient no trouble (fig. 1).

Microscopic Examination.—Sections of the lymph nodes fixed in Zenker's fluid were stained with the hemalum eosin, Van Gieson, Mallory phosphotungstic acid

hematoxylin, elastic tissue and iron stains. The histologic appearance of all the excised lymph nodes is the same. The greater part of the center of each node is composed of tumor tissue (fig. 2). In the periphery of the node a rim of intact lymphatic tissue is seen which is slightly compressed but otherwise shows no changes. The centrally located tumor tissue is separated from this peripheral rim by a narrow band of dense connective tissue. The tumor tissue making up the center of the lymph node is composed uniformly of narrow vascular spaces lined by flat endothelium. These spaces are about the diameter of small capillaries and contain red blood cells. Between the vascular spaces is a delicate stroma.

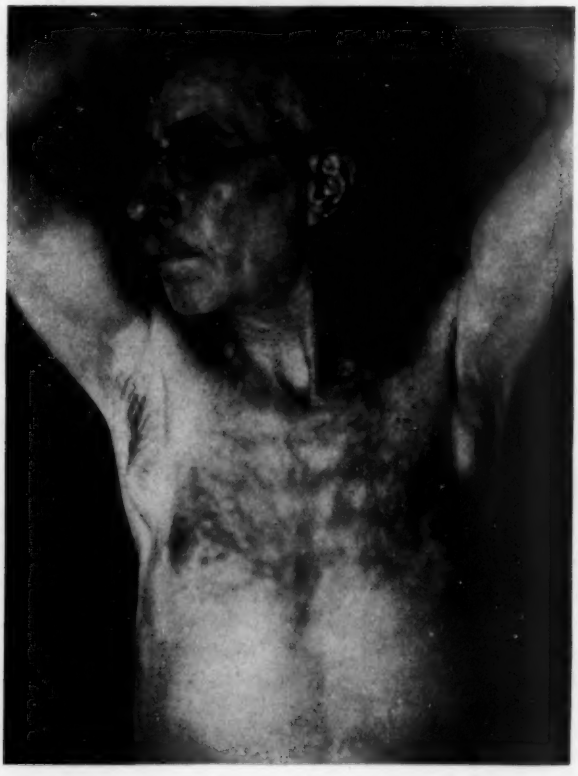

Fig. 1.—Recent photograph of the patient showing enlarged axillary, pectoral, cervical and epitrochlear lymph nodes. The biopsy scar in the right epitrochlear region is visible.

This stroma is composed of fusiform and spindle-shaped cells with elongated dark-staining nuclei. Scattered throughout are a few delicate collagen fibrils. The stroma shows intimate relations to the vascular spaces. In many places these vascular spaces can be seen in the process of formation as young capillary twigs budding from the fusiform cells. There is little anaplasia, and only occasional mitotic figures can be seen. The vasoformative character of the undifferentiated stroma composed of fusiform cells and its relation to the vascular spaces are clearly seen in all the nodes examined (fig. 3).

COMMENT

Several interesting questions present themselves: First is the possibility of the condition being one of multiple metastases. The absence of a primary source and the absence of visceral and skeletal metastases

Fig. 2.—Very low power view of a lymph node showing centrally located tumor tissue and a peripheral rim of lymphatic tissue separated from the tumor by the latter's narrow connective tissue capsule.

speak against this. Such extensive multiple metastases to lymph nodes would be exceedingly rare. Of course the possibility still exists, because, after all, only biopsy material was studied. But it is unlikely since exhaustive clinical investigation of the patient failed to reveal a primary tumor.

Second is the relationship of the tumor to the lymph nodes themselves. There seems to be no relation to the lymphatic tissue proper. The tumor is separated from the lymphatic tissue by a narrow band of collagen fibers and fibrocytes. Some other structure in the lymph node seems to have been the point of origin. Apparently vascular anlagen in

Fig. 3.—High power view of the tumor tissue (hemalum and eosin). Capillary spaces are seen budding from the fusiform cells of the stroma.

either the capsule, the trabeculae or the hilus, or the preexisting blood vessels of the lymph node, may account for the histogenesis.

Third, the possible relationship to the reticulo-endothelial apparatus of the lymph nodes must be considered. There seems to be no relationship. The tumor has distinctly the character of angiosarcoma. The

fusiform stroma cells are of differentiated angioblastic type and have no resemblance to, or relationship with, the reticulum cells. The vascular spaces are lined by ordinary vascular endothelium, which, as is well known, has no relationship to reticulo-endothelium.

The question of the degree of malignancy of the tumor is interesting. Since there is so little cellular anaplasia, since the patient's condition is still good, and since the lymph nodes have grown only very slowly in the past thirty-two months the degree of malignancy seems to be low. One is reminded of Kaufmann's case of fifteen years' duration, and of Kaposi's multiple angiosarcomatosis of the skin in which durations up to twenty-five years have been reported.

The impression in this case is that the tumors have arisen from independent multiple angioblastomatous anlagen in the lymph nodes. Such multiple systemic angiomatosis is not at all rare. Osler's disease, or multiple telangiectasia of the skin and internal organs, for instance, is well known. This hereditary condition has been traced in about one hundred families known to have the disease. It is characterized by the occurrence of multiple discrete hemangiomas in the skin and internal organs. Jaffé [4] and Goldstein [5] have made important studies of this condition. It illustrates the occurrence of multiple discrete angiomatous anlagen.

Of much greater resemblance to the present case, however, is Kaposi's multiple angiosarcomatosis of the skin. The histologic picture of this lesion is characteristic. In the early lesions ovai to spindle-shaped cells appear and proliferate, and forms in transition from them up to well formed capillary-like vascular spaces are seen. The fusiform cells produce a vasoformative tissue from which the small capillary buds grow. Dörffel [6] has made an extensive histologic study of sixteen cases of Kaposi's sarcomatosis and finds this histologic picture to be characteristic. In the late stages this picture is changed and obscured by hemorrhage and degenerative changes. Gans [7] in his textbook gives the same characteristics. Hamdi and Resat [8] found the same histologic picture in the early lesions in several cases. They also proved that the lesions are discrete and independent of each other by making serial sections through a number.

The striking similarity of the histologic picture in the present case to that of Kaposi's sarcomatosis is of more than passing interest. In

4. Jaffé, R. H.: Arch. Path. **7**:44, 1929.

5. Goldstein, H. I.: Arch. Dermat. & Syph. **26**:282, 1933.

6. Dörffel, J.: Arch. Dermat. & Syph. **26**:608, 1932.

7. Gans, O.: Histologie der Hautkrankheiten, Berlin, Julius Springer, 1928, vol. 2, p. 463.

8. Hamdi, H., and Resat, H.: Ann. d'anat. path. **9**:593, 1932.

both, the tumor tissue is vasoformative, that is, composed of undifferentiated fusiform mesenchymatous cells from which capillary buds grow. Orsos [9] has made a histologic study of eleven cases of such capillary angioblastic tumors. They are quite different from angioendotheliomas; in the latter the differentiated endothelial cells lining the vascular space are the proliferating units.

SUMMARY

The case reported here is one of generalized primary angiosarcomatosis of the lymph nodes of thirty-two months' duration in a 64 year old white man. Biopsies of four different lymph nodes revealed the same picture, namely, that of an angioblastomatous tumor of low grade malignancy, centrally located in the lymph nodes, fairly well encapsulated and apparently independent of the lymphatic tissue or the reticulo-endothelial apparatus. The origin seems to be from multiple vasoformative anlagen in the lymph nodes. The relationship to other forms of multiple angiomatosis and to Kaposi's multiple angiosarcomatosis of the skin is discussed. Two similar cases of generalized primary sarcomatosis of the lymph nodes have previously been reported.

9. Orsos, F.: Beitr. z. path. Anat. u. z. allg. Path. **93**:121, 1934.

General Review

TRYPANOCIDAL ACTION OF NORMAL HUMAN SERUM

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NEW YORK

The trypanocidal property of human serum has been known since 1902, when Laveran [1] discovered the phenomenon while studying mice experimentally infected with the trypanosome of nagana, Trypanosoma Brucei. In the years following this observation, the investigations in the field, which have taken numerous directions, have gradually indicated that this property of the serum is of considerable significance in immunology. Although a large number of papers have been presented on the subject, no general summary of the trypanocidal phenomenon has appeared in recent years, covering the various aspects which research on this interesting and striking characteristic of human serum has taken. It is to supply this need for the increasing number of workers in this and related fields that the present review is offered.

When studied with the purpose to explain the development of natural antibodies in general, the trypanocidal property of human serum takes on added significance. The average person probably at no time in his life is exposed to subclinical or latent infection or to specific immunization by other channels with any form of trypanosome. Yet, regularly, this antagonistic property develops in the blood of normal human beings soon after birth. The chance of heterologous immunization with some other agent which possesses antigenic moieties in common with the trypanosome remains, however, although as yet no such agent has been determined. It seems possible that the trypanocidal substance occurs in consonance with the normal physiologic maturation of the human body [2] and is not to be regarded as the product of a process of antibody formation. In the light of recent observations, it is perhaps not too much to hypothesize that analogous processes are responsible for the appearance in the serum of normal persons of antagonistic substances of a broad range, including those which destroy certain bacteria and certain filtrable viruses.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

1. Laveran, A.: Compt. rend. Acad. d. sc. **134**:735, 1902.
2. Jungeblut, C. W., and Engle, E. T.: J. A. M. A. **99**:2091, 1932.

In reviewing the subject, I shall discuss the limitations and the general properties of the trypanocidal substance, its formation by the body, its mode of action on trypanosomes, its correlation with man's immunity against trypanosomes, its possible clinical significance and, finally, its relationship to certain other protective activities of the serum.

LIMITATIONS OF THE TRYPANOCIDAL PROPERTY

Species of Trypanosomes Affected.—Human serum manifests its activity not only on the trypanosome of nagana (T. Brucei) but also on the trypanosomes of surra (T. Evansi), mal de caderas (T. equinum) and dourine (T. equiperdum) as well as on other trypanosomes naturally pathogenic for animals.[3] Normally, these trypanosomes possess great virulence for mice and rats, and such animals generally die in from three to five days after the parasites have appeared in the blood stream.[4] When 1 cc. of human serum is introduced into the infected animals, however, the parasites, which may have been present in tremendous numbers previously, disappear completely from the blood within a few hours. After an interval of from eight to twenty days, the trypanosomes return and, unless more human serum is introduced, promptly cause the death of the animal. Such removal of the parasites from the circulation can be accomplished repeatedly over a period of two or three months, but finally the effectiveness of the human serum is lost and the animal dies. Laveran[5] noted that only very seldom was complete cure or sterilization possible through the administration of human serum. Nattan-Larrier and Noyer,[6] likewise, frequently observed relapses after the injection of serum, and Adams[7] failed to effect cure at any time, although the temporary removal of parasites from the blood was accomplished repeatedly. The use of human serum is more successful for the purpose of preventing than of treating infections with the pathogenic trypanosomes.[8] However, amounts as small as 0.1 cc. of normal serum injected subcutaneously into a mouse of 20 Gm. weight at the time of infection with T. Brucei often are capable of causing complete inhibition of the infection.

A very striking difference in the effect of human serum is seen in mice infected with the pathogenic trypanosomes of man, T. gambiense,

3. (*a*) Laveran, A.: Compt rend. Acad. d. sc. **137**:15, 1903. (*b*) Thirouz, A., and d'Anfreville, L.: ibid. **147**:462, 1908. Laveran.[1]

4. Taliaferro, W. H.: Quart. Rev. Biol. **1**:246, 1926.

5. Laveran, A.: Compt. rend. Acad. d. sc. **138**:450, 1904.

6. Nattan-Larrier, L., and Noyer, B.: Compt. rend. Soc. de biol. **104**:475, 1930.

7. Adams, P.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **58**:456, 1928.

8. (*a*) Goebel, O.: Ann. Inst. Pasteur **21**:882, 1907. (*b*) Rosenthal, F., and Krueger, M.: Berl. klin. Wchnschr. **58**:382, 1921.

T. rhodesiense and T. Cruzi.[9] If used in the test soon after their isolation from human beings with trypanosomiasis, these parasites are insusceptible to the action of human serum. Somewhat readily, however, T. rhodesiense, in distinction from the other human forms, becomes susceptible to the effects of human serum after repeated passage through mice, rats or guinea-pigs.[10] Corson[11] has found that human serum is more effective in mouse infections with T. rhodesiense if the parasites are first passed through sheep and goats. Mesnil[12] reported that a strain of T. gambiense also became susceptible to human serum after being passed through animals in his laboratory for nine years, but another strain kept even longer, twelve years, by Laveran[13] retained throughout this period its original serum resistance. This selectivity in the action of human serum is considered by some to be of importance in connection with man's immunity to the animal pathogens and his susceptibility to the human species of trypanosomes. More will be said on this point later.

The common, nonpathogenic parasite of rats, Trypanosoma Lewisi, is also insusceptible to the trypanocidal effect of human serum. This was noted by Laveran and Mesnil[14] and recorded in one of their earliest papers. I[15] also have found T. Lewisi infections in rats unaltered in any phase of their course[16] by the administration of human serum. The resistance of T. Lewisi to human serum parallels its resistance to most of the chemicals which act so positively on the pathogenic trypanosomes.

I have found recently that Trypanosoma diemyctyli, the trypanosome of the red-spotted newt, Triturus viridescens, also is resistant to fresh human serum even when the newts are kept at a temperature of 37 C. after the injection of the serum.

Type of Serums Which Exhibit Trypanocidal Power.—The trypanocidal power of serum is not widespread among the species of animals. It is, indeed, almost exclusively a property of the serum of man. The serums of some kinds of monkeys are active, however. Large doses

9. (*a*) Laveran, A., and Nattan-Larrier, L.: Compt. rend. Acad. d. sc. **154:** 18, 1912. (*b*) Zeiss, H.: Arch. f. Schiffs- u. Tropen-Hyg. **24:**73, 1920. Laveran.[5]

10. Mesnil, F., and Ringenbach, J.: (*a*) Compt. rend. Acad. d. sc. **153:**1097, 1911; (*b*) ibid. **155:**78, 1912; (*c*) Bull. Soc. path. exot. **7:**612, 1914. (*d*) Fairbairn, H.: Ann. Trop. Med. **27:**251, 1933.

11. Corson, J. F.: J. Trop. Med. **34:**81, 1931.

12. Mesnil, F.: Compt. rend. Soc. de biol. **77:**564, 1914.

13. Laveran, A.: Bull. Soc. path. exot. **8:**442, 1915.

14. Laveran, A., and Mesnil, F.: Ann. Inst. Pasteur **18:**785, 1902.

15. Culbertson, J. T.: Ann. Trop. Med. **28:**93, 1934.

16. Taliaferro, W. H.: J. Exper. Med. **39:**171, 1924.

of the serum of the baboon, which is naturally immune to infection with all species of trypanosomes,[17] is effective even against the human forms, T. gambiense and T. rhodesiense, which are resistant to human serum.[18] In its action on the animal pathogens (T. Brucei, T. equiperdum, etc.), on the other hand, baboon serum is less powerful than human serum.[19] The serums of mangabeys and mandrills also exhibit limited activity against T. Brucei.[20] In contrast to these positive effects with the serums of certain Primates, no activity against any species of trypanosome has been observed with the serum of any of the common domestic or laboratory animals (horse, cow, sheep, goat, pig, rabbit, guinea-pig, rat, mouse and fowl).

Hosts in Which the Trypanocidal Effect of Human Serum Is Manifested.—The earliest work on the trypanocidal activity of human serum was carried out in mice and rats. Goebel,[8a] and more recently Peruzzi,[19] employed guinea-pigs in some of their tests, but the results obtained have been somewhat less constant than those obtained with mice and rats. Larger animals have been employed but rarely, probably because of the proportionately greater volume of human serum required to demonstrate the trypanocidal effect.

PROPERTIES AND NATURE OF THE TRYPANOCIDAL SUBSTANCE

Authors are in general agreement concerning many of the basic properties of the trypanocidal substance in human serum, but observations on some other characters are in such conflict that conclusions are reached only with difficulty. It is established that the trypanocidal substance is found in the fluid and not in the formed part of the blood.[22] Goebel [8a] early associated the substance with the globulin fraction of the serum, and this has found confirmation with Rosenthal and Freund [23] and others. The substance is insoluble in ether.[24] It is thermolabile: heating at 64 C. for an hour destroys wholly the activity of the serum, and exposure at a temperature of 56 C. for this interval leads to its

17. Zschucke, J.: Arch. f. Schiffs- u. Tropen-Hyg. **37**:194, 1933. Regendanz, P.: ibid. **37**:195, 1933.

18. Laveran, A.: Compt. rend. Acad. d. sc. **139**:177, 1904. Mesnil and Ringenbach.[10a]

19. Peruzzi, M., in Final Report of League of Nations International Commission on Human Trypanosomiasis, Boston, World Peace Foundation, 1928.

20. Mesnil, F., and Leboeuf, A.: Compt. rend. Soc. de biol. **69**:382, 1910.

22. (*a*) Citron, H.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **27**:369, 1918. (*b*) Rosenthal, F.: ibid. **62**:464, 1929. (*c*) Pfannenstiel, W., and Scharlau, B.: Centralbl. f. Bakt. (Abt. 1) **110**:84, 1929.

23. Rosenthal, F., and Freund, R.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **37**:48, 1923.

24. Jacoby, M.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **2**:689, 1909.

partial inactivation.[25] The addition of fresh guinea-pig serum fails to restore the original trypanocidal potency of a serum rendered inactive by heat.[26] When allowed to stand at room temperature, human serum gradually loses its trypanocidal power and in two or three months is totally devoid of activity.[3a] Even after a few days in the icebox a significant loss is noted, and after from two to four weeks marked reduction in the power is observed.[7] If, however, the serum is dried and the powder dissolved in a suitable fluid when needed, activity is retained more or less quantitatively for at least six months.[3a]

Relationship to the Serum Antibodies.—Whether or not the trypanocidal substance is identical with the usual serum antibodies is not established. Laveran and Mesnil [14] and Goebel [8a] believed it unlike an opsonin, and others [27] have considered it different from any of the usual serum antibodies. Laveran and Mesnil [14] found that human serum failed to agglutinate trypanosomes whereas goat or sheep or especially pig serum—all of which are entirely without trypanocidal action—gave marked agglutination. They also discovered that fowls, which are refractory to trypanosomes, yield, after long immunization with a suspension of trypanosomes, a serum devoid of trypanocidal property. At the same time, however, these workers found that the serum of goats or sheep immunized against trypanosomes prevented infection in mice if mixed with the trypanosomes when these were injected, although if the immune serum was inoculated elsewhere in the animal at the time of infection no protection resulted. On the other hand, Strong and I [28] observed that human serum loses its trypanocidal power after absorption with a mass of trypanosomes as well as with a mass of certain species of bacteria (e. g., the typhoid bacillus and Proteus). Hence we concluded that the trypanocidal substance is a relatively nonspecific antibody.

Relation to Alexin.—It was postulated by A. R. D. Adams [25] that "complement is the trypanocidal substance" because both properties were destroyed by heating, filtering or ammonifying a serum. In support of this, it has been found that both serum properties are lost or reduced simultaneously in certain types of disease.[29] Goebel,[8a] however, has thought the trypanocidal substance distinct from complement or alexin since the trypanocidal power, once inactivated by heating, cannot

25. Adams, A. R. D.: Ann. Trop. Med. **25**:299, 1931. Adams.[7] Goebel.[8a] Laveran and Mesnil.[14]

26. Adams.[7] Goebel.[8a]

27. Rosenthal, F., and Freund, R.: Ztschr. f. Hyg. u. Infektionskr. **97**:137, 1922. Adams.[7]

28. Culbertson, J. T., and Strong, P. S.: Am. J. Hyg. **21**:1, 1935.

29. (*a*) Lange: Klin. Wchnschr. **1**:1040, 1922. (*b*) Freund, R., and Gassmann: ibid. **8**:233, 1929.

be reactivated by the addition of fresh guinea-pig serum. Furthermore, it was pointed out that the trypanocidal action is a characteristic of the blood only of man and several kinds of apes whereas alexin is common to all forms.[30] Handler [31] has reported that no more than a crude parallelism exists between the rates of inactivation of the trypanocidal substance and that of alexin when fresh human serum is permitted to stand in the refrigerator. Strong and I [28] in studies in vitro have found that the trypanocidal substance is separable from all of the known components of alexin both by filtration through Berkefeld candles and by the specific inactivation of each of the components of alexin. Furthermore, we have succeeded in totally removing the trypanocidal substance from the serum by absorption at 0 C. with typhoid bacilli without reducing significantly the potency of the alexin. We have concluded not only that the trypanocidal substance is distinct from alexin but that each of these serum properties can manifest its activity in the complete absence of the other.

Antigenic Property of the Trypanocidal Substance.—The antigenicity of the trypanocidal substance has been a point of special interest. Laveran and Mesnil [14] offered an experiment from which they concluded that no antitrypanocidal antibody was formed as the result of repeatedly treating an animal with a trypanocidal serum. An infected rat was given seven injections of 2 cc. of human serum at the rate of one every second day, beginning two days after infection with T. Brucei. The trypanosomes were not affected by the last injection of the series, and the rat's blood was drawn. When 0.5 cc. of this blood was mixed with 0.5 cc. of human serum and this mixture injected into an infected rat, the trypanocidal action of the human serum was still demonstrable. Goebel,[8a] on the other hand, found that human serum after being mixed with rabbit antihuman serum lost part of its curative power and was no longer protective. Rosenthal and Freund [23] explained the gradual loss of trypanocidal power after repeated injections of human serum, not on the basis of antibody formation, but by an "exhaustion" mechanism, which will be discussed later. Recently, Handler [31] presented what is perhaps the most satisfactory evidence on the problem of antigenicity of the trypanocidal substance of serum. He concluded that the trypanocidal substance has the capacity to produce an equivalent precipitating antibody separate from those formed against other proteins of the serum, and has been able completely to inactivate in vitro the trypanocidal substance in human serum by precipitation with an optimum amount of a specific immune serum from the rabbit. Neither the supernatant fluid over the precipitate nor the precipitated

30. Rosenthal, F.: Klin. Wchnschr. **3**:1657, 1924. Rosenthal and Freund.[27]

31. Handler, B. J.: Am. J. Hyg. **21**:18, 1935.

substance manifested trypanocidal activity. Handler [31] considered the trypanocidal substance antigenically distinct from other fractions of the human serum because an immune serum prepared against a human serum in which the trypanocidal substance has been inactivated by heat was incapable of neutralizing the trypanocidal substance of a fresh human serum. The same author demonstrated similar antagonistic effects in vivo by treating infected mice with human serum and, separately, with rabbit antihuman serum. It seems probable from this that the trypanocidal substance of the human serum is antigenic and gives rise to a specific neutralizing antibody on injection into animals. The inability of some earlier workers to demonstrate the inhibitory effect of antihuman serum on the trypanocidal power of normal human serum probably lies in their failure to observe necessary quantitative relationships between the antigen and the antibody concerned.

Additional Properties.—A number of other properties of the trypanocidal substance should be mentioned. Adams [7] found the trypanocidal action more marked when blood was permitted to clot at 37 C. or at room temperature than when placed at 0 C. With him, serums that gave a positive Wassermann reaction exhibited a greater trypanocidal power than those that gave a negative Wassermann reaction. Laveran and Mesnil [14] believed that the rapidity of the removal of parasites depended on the number present, but noted that frequently the injection of 0.5 cc. of serum was more effective than the administration of twice this quantity. Johnson,[32] however, has been unable to demonstrate any such zone phenomenon in connection with the action of human serum. The trypanocidal substance passes readily through Berkefeld candles,[28] although it is partly lost by ultrafiltration,[33] the latter property again differentiating it from alexin. The trypanocidal substance probably does not pass the placenta.[34]

FORMATION OF THE TRYPANOCIDAL SUBSTANCE IN THE HUMAN BODY

One of the early observations of Laveran and Mesnil [14] was that while the serum of a human being exhibited marked trypanocidal power, the pleural fluid, ascitic fluid and possibly even the blood plasma showed much less activity. It was thought, therefore, that the trypanocidal substance emanated from the leukocytes and was freed or separated from them during blood coagulation. Body fluids which possessed few leukocytes would, accordingly, be potentially poor in trypanocidal power.

32. Johnson, T. L.: Am. J. Hyg. **9**:260 and 283, 1929.

33. Nattan-Larrier, L., and Noyer, B.: Compt. rend. Soc. de biol. **105**:630, 1930.

34. Nattan-Larrier, L., and Lépine, P.: Compt. rend. Soc. de biol. **97**:1470, 1927.

Salmon,[35] however, injected the leukocytes from two kinds of pus as well as an extract of a lymphatic gland without eliciting the trypanocidal action. When he tried serous exudates, he found, in contrast to the results of Laveran and Mesnil,[14] marked trypanocidal effects. Salmon [35] decided, therefore, that the substance was a property of the blood fluid and not of any of the blood cells. This has been confirmed by Rosenthal [22b] and Pfannenstiel and Scharlau.[22c] Salmon [35] and Regendanz [36] have found cerebrospinal fluid inactive. The urine has no trypanocidal power.[37] Rosenthal [22b] also has found emulsions of various organs inactive.

The Liver as the Site of Formation of the Trypanocidal Substance.—Several investigators have noted that in the serums of patients suffering from certain types of liver disorders the trypanocidal activity is sharply reduced.[38] Rosenthal [30] and Rosenthal and Nossen [39] concluded that the trypanocidal substance found in human serum is a product of the normally functioning healthy liver and is decreased in amount as certain pathologic conditions disturb this normal function. A large amount of important work in connection with the diagnosis of liver disorders has resulted. As the problem now stands, there is general agreement with Rosenthal that the trypanocidal substance is formed by the healthy liver.

Appearance of the Trypanocidal Property in Infants.—It is recognized that the serum of very young infants is less active in trypanocidal power than is that of adults.[40] Neumark and Pogorschelsky [40b] believed the trypanocidal substance was absent in children up to the age of 3 weeks and gradually increased during the first three months of life. Levy,[40c] however, has found the substance in a large percentage of the serums of children 3 weeks of age, some of whom unfortunately could not be considered to be in normal good health, and Nattan-Larrier and Lépine [34] have detected it even in the blood of a new-born infant. Nattan-Larrier and Lépine [34] believed that the activity of the serum of very young children was not due to passage of the trypanocidal substance through the placenta but to the precocious development of this substance in the young child. Rosenthal and Kleeman [40a] and others [41]

35. Salmon, P.: Bull. Soc. path. exot. **3**:726, 1910.

36. Regendanz, P.: Zentralbl. f. Bakt. (Abt. 1) **120**:89, 1931.

37. Rosenthal.[22b] Salmon.[35]

38 (*a*) Ehrlich, P.: Berl. klin. Wchnschr. **44**:233, 1907. (*b*) Platau, L.: Ztschr. f. Hyg. u. Infektionskr. **81**:401, 1916. Rosenthal and Krueger.[8b]

39. Rosenthal, F., and Nossen, H.: Berl. klin. Wchnschr. **58**:1093, 1921.

40 (*a*) Rosenthal, F., and Kleeman, E.: Berl. klin. Wchnschr. **52**:75, 1915. (*b*) Neumark, E., and Pogorschelsky, H.: Klin. Wchnschr. **4**:1725, 1925. (*c*) Levy, S.: Jahrb. f. Kinderh. **120**:325, 1928. Laveran.[3a] Nattan-Larrier and Lépine.[34]

41. Eufinger, H.; Rothemundt, M., and Wiesbader, H.: Monatschr. f. Geburtsh. u. Gynäk. **93**:249, 1933.

reported an increase in the trypanocidal power of the serum of pregnant women in the last part of the gestation period, and Neumann [42] noted that the serum of pregnant women was powerfully trypanocidal directly after term. Rosenthal and Kleeman [40a] obtained no evidence for the transmission of the substance from the mother to the child during nursing since the milk lacked trypanocidal activity.

The trypanocidal potency of the serum of children is subject to more marked fluctuation than is that of adults. This has been explained by assuming the building up of a store of the substance in the fluids and tissues as the child becomes older.[43] Rosenthal and Nossen [39] believe that the lack of the substance in very young children is due to the incomplete functioning of the organs of the young child.

A considerable variation occurs in the trypanocidal potency of the normal serums of healthy adult persons.[44] Whether there is a relationship between the concentration of this substance and the human blood groups is not as yet indicated. This point may deserve investigation since somewhat greater resistance to certain diseases has been reported in persons of certain blood groups.[45]

MODE OF ACTION OF THE TRYPANOCIDAL SUBSTANCE ON THE PARASITE

The mode of action of the trypanocidal substance on trypanosomes is not well understood. Some have felt that it behaves as an antibody, possibly requiring the presence of alexin for the manifestation of its activity. Others believe the trypanocidal substance acts essentially as a chemotherapeutic agent, perhaps complemented by some element elaborated in the body of the animal in which the trypanocidal action takes place. The development by Yorke and his co-workers [46] of a method for demonstrating the trypanocidal effect of serum wholly in vitro has greatly facilitated study of the problem and has made possible the correction of certain faults in theory which previously had been accepted.

The Trypanocidal Substance as an Antibody.—There is no reason to believe that the trypanocidal action is opsonic.[47] It was early recognized that phagocytosis of the living parasites was not stimulated as a result of administering human serum, although leukocytes were observed

42. Neumann, R.: Ztschr. f. Hyg. u. Infektionskr. **69**:109, 1911.

43. Grünmandl, S., and Leichtentritt, B.: Jahrb. f. Kinderh. **106**:203, 1924. Rosenthal and Nossen.[39]

44. Laveran and Mesnil.[14] Platau.[38b]

45. Lattes, L.: Individuality of the Blood in Biology and in Clinical and Forensic Medicine, New York, Oxford University Press, 1932.

46. Yorke, W.; Adams, A. R. D., and Murgatroyd, F.: Ann. Trop. Med. **23**:501, 1929.

47. Laveran.[1] Goebel.[8a] Laveran and Mesnil.[14]

to engulf dead trypanosomes in the blood stream of a serum-treated mouse. Rosenthal and Spitzer [48] found the trypanocidal power not reduced in aleukocytic animals—that is, animals treated with thorium-x to reduce the number of leukocytes. There seems at present no reason to believe that either leukocytes or opsonins are of importance in the trypanocidal activity of human serum.

Considerable debate has occurred as to whether or not the mechanism of action of the trypanocidal substance is the same as that of alexin and sensitizer. Goebel [8a] insisted that serum acted neither preventively nor curatively by such a mechanism and believed that the loss of activity by heating, aging and treating the serum with alkali—which appeared to favor the function of such a mechanism—could be explained otherwise than by inactivation of alexin. Goebel obtained no fixation in vitro by trypanosomes of any substance to which the serum owed its activity. With him, human serum digested at 37 C. with trypanosomes retained its preventive and curative properties, and the exposed parasites likewise retained their infectivity. Because of his failure to effect fixation of the trypanocidal substance to either trypanosomes or yeast cells (the latter of which had been shown by von Dungern [49] to fix alexin) and because of his inability to obtain reactivation with fresh guinea-pig serum, Goebel [8a] concluded that the trypanocidal activity was due to something in the serum other than alexin. Recently, Rosenthal,[22b] in agreement with Goebel,[8a] found no fixation of alexin in vitro and considered the trypanocidal action unlike that of any of the usual serum antibodies, a point to which Adams [7] also agreed. Apparently the reaction between normal human serum and susceptible trypanosomes is unlike that occurring between an extract of trypanosomes and the serum of a recovered or an infected animal, because fixation occurs in the latter case. Indeed, the test for alexin fixation has had considerable use in the diagnosis of trypanosome infection among animals [50] as well as in the classification of the trypanosomes.[51] Furthermore, complement-fixing antibodies of rather limited specificity can be demonstrated in the serum of guinea-pigs either after injection of dead trypanosomes or after infection with the parasites.

The Trypanocidal Substance as a Chemotherapeutic Agent—The Exhaustion Phenomenon.—Rosenthal [52] recently has concluded that the

48. Rosenthal, F., and Spitzer, F.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **40**:529, 1924.

49. von Dungern, F.: München. med. Wchnschr. **47**:677, 1900.

50. Levaditi, C., and Mutermilch, S.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **2**:702, 1909. Schoening, H. W.: J. Infect. Dis. **34**:608, 1924. Landsteiner, K., and van der Scheer, J.: J. Exper. Med. **45**:465, 1927.

51. Robinson, E. M.: Eleventh and Twelfth Report, Director, Vet. Educ. & Res., Dept. Agric., Union of South Africa, September 1926, p. 9.

52. Rosenthal, F.: Med. Klin. **26**:205, 1930.

mode of action of human serum on trypanosomes is analogous to that of a true chemotherapeutic substance. Since he was unable to demonstrate any trypanocidal action in vitro he concluded that human serum was not itself trypanocidal but was rather trypanocidogenic. He thought the trypanocidogenic substance of human serum was transformed by ferments of the blood into the essential trypanocidal material in a manner somewhat analogous to the supposed mode of action of the trypanocidal chemical atoxyl. Atoxyl, in its usual form, is not lethal for trypanosomes in vitro, but after injection into the animal it is, in some fashion, transformed into an active product. Ehrlich [53] considered that a reduction of the chemical occurred in the animal body, and substances have been demonstrated in the blood [54] and liver cells [55] which are capable of effecting such a change of atoxyl in the test tube.

In an effort to demonstrate ferments which might be responsible for the transformation of the trypanocidogenic substances of human serum into the essential trypanocidal material, Rosenthal and Spitzer [48] blocked the reticulo-endothelial system with india ink and removed the spleen of infected rats prior to injection of human serum. Either procedure decreased the effectiveness of the injected serum. When the reticulo-endothelial system was blocked and splenectomy was done prior to the injection of the serum, very little trypanocidal effect was observed. This appeared to round out well the theory of Rosenthal to explain the mode of action of human serum. Briefly, then, according to Rosenthal, human serum seems to contain trypanocidogenic materials which are transformed into trypanocidal substances by ferments arising from or contained in the reticulo-endothelial system of the treated animal. The final loss of effectivity of the human serum in mice repeatedly treated with this substance Rosenthal and Freund [23] would explain by the exhaustion from the reticulo-endothelial system of the ferments necessary for this conversion. As yet, however, Rosenthal has not been able to demonstrate the existence of substances which can modify the human serum in vitro in a manner analogous to that seen with atoxyl.

Recently, however, Handler's [31] work has thrown some question on the mechanism of the trypanocidal action as advanced by Rosenthal. He has been able to produce "exhaustion" with a human serum which had been heated at 64 C. for an hour in order to destroy its efficacy as a trypanocide. Handler explains the loss of effectiveness of human serum in animals which have been repeatedly treated with the substance on the basis of specific antibody production. This he has been able to show rather conclusively by injecting an antihuman serum from the

53. Ehrlich, P.: Verhandl. d. deutsch. dermat. Gesellsch. **10**:52, 1908.

54. Yamanouchi, T.: Compt. rend. Soc. de biol. **68**:120, 1910.

55. Levaditi, C.: Ann. Inst. Pasteur **28**:604, 1909.

rabbit into trypanosome-infected mice simultaneously with an active trypanocidal human serum. When a considerable amount of a potent antiserum is employed, the trypanocidal activity of the human serum can thus be wholly overcome. Zimmermann [56] also has opposed the exhaustion theory since by increasing the amount of human serum injected into an "exhausted" animal—thereby perhaps supplying sufficient antigenic material to neutralize the corresponding antibody present in the mouse circulation—trypanocidal effects could be elicited.

One of the important points on which the "exhaustion theory" of Rosenthal was based was his belief that human serum was without activity in vitro. Recent work, however, has shown conclusively that no support can be given the exhaustion theory from this quarter. Following the earlier work of Schern,[57] Terry [58] and others, Yorke, Adams and Murgatroyd [46] perfected a method for maintaining pathogenic trypanosomes alive quantitatively in vitro at 37 C. for twenty-four hours. In contrast to the earlier workers, they found that human serum is highly trypanocidal in vitro, dilutions as great as from 1 : 5,000 to 1 : 25,000 often being active against a strain of T. equiperdum as well as against an old strain of T. rhodesiense which they employed. Apparently, there exists no essential difference between the mode of action of the human serum in vitro and in vivo, and the hypothesis of specific ferments for modifying the human serum after its injection into an animal is groundless. As with the tests in the animal body, no action of human serum on T. gambiense is demonstrated in vitro, and serums from persons suffering from certain pathologic processes (amebic abscess of the liver and obstructive jaundice) show little or no trypanocidal power by the in vitro test. The same workers [59] have made the interesting observation that the normal serums of certain sheep and goats are naturally antitrypanocidal since often a trypanocidal human serum becomes wholly inactive, as determined by the in vitro test, after being mixed with a small amount of the serum of a given sheep or goat.

Prior to this publication by Yorke and his group on in vitro action of human serum, the only statement of the successful use of human serum in vitro was that by Saito [60] in 1927. Laveran and Mesnil,[14] Goebel [8a] and Rosenthal [61] had failed to obtain the trypanocidal effect outside the animal body. Since the publication by Yorke and his group,

56. Zimmermann, G.: Zentralbl. f. Bakt. (Abt. 1) **120**:422, 1931.

57. Schern: Arb. a. d. k. Gsndhtsamte **38**:338, 1911.

58. Terry, B. T.: Proc. Soc. Exper. Biol. & Med. **9**:41, 1911.

59. Yorke, W.; Adams, A. R. D., and Murgatroyd, F.: Ann. Trop. Med. **24**: 115, 1930.

60. Saito, M.: Fukuoka-Ikwadaigaku-Zasshi **20**:52, 1927.

61. Rosenthal.[22b, 30, 52]

numerous workers in different laboratories have reported positive results in in vitro tests and, although the test requires extremely delicate adjustment and tedious attention to details, there remains no question of the success of the method.[62] Adams [25] has noted not only with human serum but with the serums of different mammals, birds and reptiles an in vitro trypanocidal effect on the gut and salivary gland forms of T. gambiense recovered from laboratory-infected tsetse flies.

Serum-Fast Strains of Trypanosomes.—As was stated in a foregoing paragraph, when a trypanosome-infected mouse has been treated repeatedly with human serum, the trypanosomes become fast or resistant to the human serum. In mice the parasites finally will resist as much as 2 cc. of fresh human serum.[63] The fastness acquired by the trypanosomes is apparently relatively permanent since the resistant character is retained by the parasites even after passage through the insect vector.[64] In this respect, serum-fastness resembles the resistance which can be developed in trypanosomes against certain drugs.[65] After repeated passage of the trypanosomes through normal animals, the acquired serum-fastness is gradually lost. For example, three races of T. Brucei which had become resistant to 2 cc. of the serum of either the baboon or man lost their refractory state after from two to fifteen passages through animals.[66] It has been feared by some that serum-fast strains might prove infective for laboratory workers, and one case of what appears to have been a laboratory infection with T. Evansi has been recorded.[67] Nevertheless, Collier [68] inoculated himself, then four other persons, with a serum-fast strain of T. Brucei but obtained infection in no instance. Likewise, Mesnil and Leboeuf [69] found that a baboon remained completely resistant to a strain of trypanosomes which was made fast to baboon serum.

Neither the "exhaustion" hypothesis of Rosenthal and Freund [23] nor the antibody production per se suggested by Handler [31] explains fully the mechanism of serum-fastness. It appears that the parasites themselves become biologically altered, particularly since the character

62. Fairbairn, H.: Ann. Trop. Med. **27**:185, 1933. Corson, J. F.: J. Trop. Med. **36**:365, 1933. Zimmermann.[36] Culbertson and Strong.[28]

63. Jacoby, M.: Med. Klin. **5**:252, 1909. Jacoby.[24]

64. Lester, H. M. O.: Ann. Trop. Med. **26**:525, 1932. Corson, J. F.: J. Trop. Med. **36**:378, 1933; **37**:113, 1934.

65. Duke, H. L.: Interim Report, League of Nations International Committee on Human Trypanosomiasis, Boston, World Peace Foundation, 1927, p. 24. Yorke, W.; Murgatroyd, F., and Hawking, F.: Brit. M. J. **1**:176, 1933.

66. Leboeuf, A.: Ann. Inst. Pasteur **25**:882, 1911.

67. Mesnil, F., and Blanchard, M.: Bull. Soc. path. exot. **7**:196, 1914. Jacoby.[63]

68. Collier, W. A.: Arch. f. Schiffs- u. Tropen-Hyg. **28**:484, 1924.

69. Mesnil, F., and Leboeuf, A.: Compt. rend. Soc. de biol. **72**:505, 1912.

of fastness persists during passage through successive vertebrate or invertebrate hosts. Evidence of similar fastness to specific serum antibodies has been presented by Massaglia [70] in experimental trypanosome infections of guinea-pigs, as well as by Novy and Knapp [71] in experimental infections with the spirochete of relapsing fever in rats.

RELATIONSHIP BETWEEN THE TRYPANOCIDAL ACTION OF HUMAN SERUM AND MAN'S SUSCEPTIBILITY TO INFECTION BY TRYPANOSOMES

It was believed by the early workers that the natural immunity of man to the trypanosomes pathogenic for animals was related to the trypanocidal power of human serum.[72] Similarly, the natural immunity of the baboon to infection with all species of trypanosomes, including those infective for man, was explained as being due to the trypanocidal substances of the baboon serum.[3a] Rosenthal,[52] on the other hand, believing the normal serum itself not to be trypanocidal but rather after injection to give rise by metabolic digestion in the mouse to trypanocidal substances, considered man's immunity due to some other agency. The older point of view has been championed anew by Yorke and his co-workers [59] largely on the basis of results with their in vitro technic.

In support of his teleologic point of view Yorke [59] has offered an interesting hypothesis concerning the relationship of the human trypanosomes to the trypanosome of nagana, T. Brucei. He has suggested, as did others before him,[73] that the human forms are modifications of T. Brucei and has postulated that if man suffers with some hepatic dysfunction or exists for a time on a diet deficient in essential accessory factors, he may become susceptible to infection with T. Brucei because the trypanocidal property disappears from his blood. Once established in such a person, the parasite may be successfully transferred to new human hosts by the natural vector, the tsetse fly. If the fly is of the species Glossina palpalis, the trypanosome becomes modified somewhat further than if the vector is of the species Glossina morsitans, since the former abounds near the dwellings of man and effects more frequent human passage of the parasite. A trypanosome which represents greater modification from the animal parasite T. Brucei is believed by

70. Massaglia, M. A.: Compt. rend. Acad. d. sc. **145**:572, 1907.

71. Novy, F. G., and Knapp, R. E.: J. Infect. Dis. **3**:291, 1906.

72. Laveran.[3a] Jacoby.[63]

73. Kinghorn, A., and Yorke, W.: Ann. Trop. Med. **6**:1, 1912. Yorke, W., and Blacklock, B.: Brit. M. J. **1**:1234, 1914. For a point of view opposite those in the foregoing references see Kleine, F. K., and Eckard: Ztschr. f. Hyg. u. Infektionskr. **75**:118, 1913. Kleine, F. K.: ibid. **77**:184, 1914. Taute: Arb. a. d. k. Gsndhtsamte. **44**:102, 1913. Fischer: Arch. f. Schiffs- u. Tropen-Hyg. **17**:621, 1913. Corson, J. F.: Ann. Trop. Med. **26**:109, 1932.

Yorke to be T. gambiense, commonly spread by G. palpalis; the human pathogen of lesser modification, T. rhodesiense, is spread by G. morsitans, which is also the vector of T. Brucei. Such a hypothesis seems to receive some substantiation from the character of T. rhodesiense, already referred to: namely, its greater susceptibility, in comparison with T. gambiense, to the action of human serum after repeated passage through laboratory animals.[74] Yet there are significant experiments which have led to conclusions which oppose the point of view that the trypanocidal activity of serum is responsible for man's immunity against the animal trypanosomes. The serum of patients suffering with trypanosomiasis is as active on the trypanosomes pathogenic for rats as is that of normal persons.[11] Some strains of guinea-pigs and white rats have been found resistant to T. Brucei despite the absence of trypanocidal substances from their blood.[75] A strain of T. rhodesiense which was rendered susceptible to human serum by repeated passage through laboratory animals was shown to retain its infectivity for man [10d] and, conversely, a strain of T. Brucei which was rendered serum-fast by repeated exposure to human serum was found unable to infect man.[68] Finally, because of the widespread distribution of the serum-resistant trypanosome, T. Lewisi,[15] among wild rats and its natural spread by means of the rat flea (Ceratophyllus fasciatus), which is known to carry certain infectious agents (e. g., Pasteurella pestis and the plague bacillus) from the rat to man, the potential incidence of human infection with this rat parasite would seem to be considerable. However, despite its resistance to human serum, T. Lewisi is apparently not infective for man, since only a single authentic case of human infection with the parasite had been reported up to 1933.[76] Because of the reasons given I am inclined to agree with Adams [77] that the immunity of man to the trypanosomes pathogenic for animals depends on other factors in addition to the trypanocidal activity of the serum.

RELATIONSHIP OF THE AMOUNT OF THE TRYPANOCIDAL SUBSTANCE IN HUMAN SERUM TO HUMAN DISEASE

Although the trypanocidal titer of normal human serum varies considerably, marked reduction or complete loss of trypanocidal activity accompanies certain diseases. In 1907, Ehrlich [88a] noticed that a disturbance of the liver led to a decrease in the amount of the trypanocidal substance of human serum, and Laveran and Nattan-Larrier,[9a] in a few trials, reported that variations from the normal strength of

74. Mesnil and Ringenbach.[10e] Corson.[11] Laveran.[13]

75. Corson, J. F.: J. Trop. Med. **36**:53, 1933.

76. Johnson, P. D.: Tr. Roy. Soc. Trop. Med. & Hyg. **26**:467, 1933.

77. Adams, A. R. D.: Ann. Trop. Med. **27**:309, 1933.

the substance occurred in the serums of persons suffering from tuberculosis or syphilis. Beginning with the work of Rosenthal and Kleeman [40a] in 1915, numerous investigations of the effects of various diseases on the amount of trypanocidal substance in the serum have been carried out. Some workers have reported a decrease in the trypanocidal potency of the serum in infectious processes, particularly when accompanied by fever,[78] usually with a return to normal on recovery,[79] but others have failed to note such modifications.[80] A sharp reduction in serum taken at the time of paroxysm from patients with dementia paralytica who were artificially infected with malaria has been reported,[81] although the serums of similar patients not so treated are said to be particularly active in trypanocidal power.[82]

A serious difficulty in the interpretation of the results of the various workers is caused by the fact that often sufficient care has not been taken to test all serums under entirely comparable circumstances. It has been shown recently, for example, by Lester [83] and by Handler [31] that the potency of serums falls rapidly in the first few days after bleeding. In comparative titrations, therefore, the interval between taking and testing the serum sample must be standardized. It is all too obvious in some cases that this necessary precaution has not been observed.

Liver Disorders.—It appears well established that parenchymal injury to the liver tissue causes a decrease in the amount of trypanocidal substance.[84] Munter [84c] believed the extent of injury to the liver was quantitatively correlated with the reduction in the amount of trypanocidal substance. According to Platau,[38b] circumscribed infection of the liver had no effect on the amount of the substance, and hepatic processes going on without icterus affected the amount of the trypanocidal substance only near their terminal stages. There is general agreement that the cure of the disorder leads to the prompt return of the trypanocidal power.[85] Rosenthal and Nossen [39] were able to differentiate, by the trypanocidal serum test, catarrhal icterus, which

78. Neumark, E., and Pogorschelsky, H.: Ztschr. f. Kinderh. **40**:535, 1926.

79. Barlowi, cited by Peutz.[84b]

80. Leichtentritt, B.: Ztschr. f. d. ges. exper. Med. **29**:658, 1922.

81. Jaffé, R. H., and Brown, S.: Proc. Soc. Exper. Biol. & Med. **19**:658, 1922.

82. Plaut: Ztschr. f. d. ges. Neurol. u. Psychiat. **101**:512, 1926.

83. Lester, H. M. O.: Ann. Trop. Med. **27**:361, 1933.

84. (*a*) Ehrlich, P.: Beiträge zur Pathologie und Chemotherapie, Leipzig, A. Pries, 1909. (*b*) Peutz, L. A.: Nederl. tijdschr. v. geneesk. **66**:1544, 1922. (*c*) Munter, F.: Klin. Wchnschr. **4**:1967, 1925. Rosenthal and Krueger.[8b] Rosenthal and Freund.[27] Ehrlich.[38a] Platau.[38b] Rosenthal and Kleeman.[40a] Eufinger et al.[41] Rosenthal.[52] Yorke et al.[59]

85. Platau.[38b] Rosenthal and Nossen.[39]

eliminated the trypanocidal power, from hemolytic icterus, which caused no loss of the substance. Yorke and his co-workers [59] demonstrated in vitro the loss of trypanocidal power in persons with amebic abscess of the liver and obstructive jaundice. Ziess [86] observed that icteric human serum is reactivated by an addition of normal human serum and suggested that the injection of normal human serum might be of help in cases of icterus. Support for the suggestion is as yet not available.

Tuberculosis.—Laveran and Nattan-Larrier [9a] found that serum from tuberculous patients in amounts of 1 cc. inoculated intraperitoneally with the infective dose of trypanosomes delayed the death of mice in two cases till the eleventh and the fourteenth days, respectively, whereas the untreated control animal died on the fifth day after inoculation. It seems unlikely from this that the trypanocidal power is significantly reduced in infections with the tubercle bacillus since normal serum inoculated in a similar amount often will inhibit infection for no longer period. Yet Laveran's conclusions are in agreement with the later work of Mignoli,[87] who noted further that the serum of tuberculous persons in progressive stages of recovery manifested increasing trypanocidal activity. The serums of two patients with Hodgkin's disease, which by some is believed caused by the avian form of the tubercle bacillus, showed no less activity against T. Brucei than did normal serum.[88]

Syphilis.—The results obtained with the serums of syphilitic patients are especially confusing since there is no consistent evidence of a marked alteration in the trypanocidal power one way or another as the result of syphilitic infection. Laveran and Nattan-Larrier,[9a] using a mixture of trypanosomes and 1 cc. of human serum from a syphilitic patient, obtained no evidence of infection in a mouse sixteen days after inoculation, whereas the control mouse not given serum was dead seven days after the inoculation. Recently, Adams [7] was able to effect complete cure in one instance, a result duplicated by him with no other human serum. The same author has found that the serum of a syphilitic patient infected also with malaria retarded the appearance of the parasites only four days; i. e., the serum apparently had lost practically all its trypanocidal power (cf. Jaffé and Brown [61]). The trypanocidal substance is said to appear precociously in congenitally syphilitic infants.[82]

Human Trypanosomiasis.—The effect of trypanosomiasis on the strength of the trypanocidal substance is of importance with respect

86. Ziess, H.: Arch. f. Schiffs- u. Tropen-Hyg. **25**:302, 1921.
87. Mignoli, A.: Riforma med. **40**:577, 1924.
88. Ziess, H.: Arch. f. Schiffs- u. Tropen-Hyg. **25**:211, 1921.

to the significance of the substance in the protection of man. Some workers have reported that both during and after recovery from trypanosomiasis the serum of patients exhibits no trypanocidal action.[9b] Recently, however, Corson [11] in tests on a strain of T. rhodesiense which had been passed repeatedly through mice obtained about as consistent action with the serums of patients infected with trypanosomes as with the serums of normal persons. If Corson's [11] observations are confirmed, it follows that the trypanocidal substance of the serum alone is insufficient to account for man's immunity to the animal trypanosomes.

Pernicious Anemia.—Frequently in cases of pernicious anemia the trypanocidal substance is reduced in amount.[89] Rosenthal [89] believed this reduction due to some secondary involvement related perhaps to impairment of normal hepatic function. When the condition of the blood becomes normal, the trypanocidal substance also returns to its normal potency.

Hemophilia.—Considerable variation in results occurs in work with the serum of hemophilic persons. Opitz and Zweig [90] found that the serums of both hemophilic children and their mothers were deficient in the trypanocidal material. Leichtentritt [91] also observed that hemophilic persons showed such a deficiency. Later, Leichtentritt and Opitz,[92] in a study on the serums of the members of a hemophilic family, found that of eight manifest bleeders the serums of seven showed no trypanocidal activity and that the serums of eighteen of nineteen nonbleeding male and female relatives likewise had no effect on infected mice. In direct contrast to these findings, however, is the work of Werner and Hartman,[93] who found that neither the plasma nor the serum of hemophilic persons or of their mothers and fathers was deficient in trypanocidal activity. Leder,[94] in a later study, reported that the serums of manifestly hemophilic persons were without trypanocidal power, whereas the serums of carriers were normal. Leder [94] believed, however, that a means of diagnosing the carrier condition might be worked out through the test for trypanocidal substance. The relation of hemophilia to the presence of trypanocidal substance is worthy of more investigation, since a means of diagnosing the carrier condition would have practical application.

89. Rosenthal, F.: Klin. Wchnschr. **8**:1436, 1929. Freund and Gassman.[29b] Rosenthal.[82]

90. Opitz, H., and Zweig, H.: Jahrb. f. Kinderh. **107**:155, 1924.

91. Leichtentritt, B.: Klin. Wchnschr. **4**:1899, 1925.

92. Leichtentritt, B., and Opitz, H.: Med. Klin. **23**:59, 1927.

93. Werner, O., and Hartman, E.: Med. Klin. **22**:1803, 1926.

94. Leder: München. med. Wchnschr. **75**:562, 1928.

Food Deficiency Diseases.—In 1922, Leichtentritt and Zielaskowski [95] observed that the serums of children suffering from Barlow's disease and from other diseases attributable to deficiencies in accessory food factors exhibited a loss of the trypanocidal substances. Grünmandl and Leichtentritt [43] since have found that the serums of children suffering from scurvy, rickets and other avitaminoses were without trypanocidal power. When the cause of the disease was eliminated by supplying the material which the diet lacked, the trypanocidal substance reappeared in the circulation. Young children appear especially suited to the study of the effect of particular diseases, especially the avitaminoses, on the trypanocidal power of the serum since children, in contrast with adults, have but little reserve store of the trypanocidal material.

In an effort to relate the trypanocidal substance with a specific factor of the diet, Jungeblut and I have tested crystalline vitamin C, or cevitamic acid, for trypanocidal activity, this substance having previously been shown by Jungeblut and Zwemer [96] to have the power to neutralize diphtheria toxin as well as the virus of poliomyelitis. The substance was, however, without trypanocidal activity under the conditions tested (5 mg. of cevitamic acid injected intravenously or 10 mg. intraperitoneally into mice for three days before infection and on each day during infection until death). The experimental mice and the control mice died after the same average number of days.

Clinical Use of the Test for the Presence of Trypanocidal Substance.—Munter [84c] believed that the degree of reduction of the trypanocidal substance in the serum so closely paralleled the extent of injury to the liver that measurement of this activity carried diagnostic and prognostic significance. Münch,[97] however, while believing the phenomenon should not be dispensed with, felt the method could hardly be used as a standard for the diagnosis of liver dysfunction. He pointed out that considerable variation occurred both in the action of normal human serums on a given strain of trypanosomes and in the resistance which different strains of trypanosomes manifested to a given human serum. Further, Münch [87] considered that a delay of from eight to fourteen days in performing the test permitted no general adoption of the method. He pointed out that methods already in use were either preferable to the test for trypanocidal substance or were at least as satisfactory for clinical purposes.

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95. Leichtentritt, B., and Zielaskowski, M.: Jahrb. f. Kinderh. **98**:310, 1922.

96. Jungeblut, C. W., and Zwemer, R. L.: Proc. Soc. Exper. Biol. & Med. **32**:1229, 1935.

97. Münch, H.: München. med. Wchnschr. **70**:945, 1923.

Certainly the objections of Münch [97] were well taken. In view of the apparently diverse diseases which affect the amount of trypanocidal substance, it seems likely the method cannot be generally adopted unless it is much further simplified. Yet work already reported by Rosenthal and his co-workers, Leichtentritt and his group, Mignoli and Yorke suggests that further investigations of possible clinical applications of the test for trypanocidal substance are indicated. Leichtentritt and Zielaskowski [95] suggested that, by aid of the trypanocidal serum test, a better understanding of the classification of diseases as well as something of the etiology of diseases the causes of which are obscure might be obtained.

RELATIONSHIP OF THE TRYPANOCIDAL SUBSTANCE TO THE BACTERICIDAL AND THE VIRUCIDAL PROPERTIES OF HUMAN SERUM

Normal human serum exhibits a destructive action on many microagents of disease. This action was first noted with respect to bacteria and later with respect to trypanosomes. In recent years, the normal human serum has been shown to possess the power to neutralize several of the filtrable viruses which attack man, particularly those of poliomyelitis [98] and herpes.[99] It seems not impossible that the agent responsible for this property of the serum is a single entity with the ability to manifest its action on a broad diversity of infectious agents. If this is true, exposure to the specific agent, as suggested by Aycock [100] and Aycock and Kramer,[101] appears unnecessary for the development of the neutralizing substance in the serum. Aycock has suggested that the immunity of adults and older children to poliomyelitis is built up by the same mechanism as that widely accepted in regard to diphtheria, namely, by exposure to a widespread or even ubiquitous disease agent and by subclinical or aborted attacks of the disease. Aycock's view has been substantiated by numerous workers.[102] Similar immunization to trypanosomes by subclinical or unrecognized attacks seems almost impossible, yet the trypanocidal substance is present universally in the blood of healthy persons. Moreover, it increases in frequency with advance of age and is present in various quantities in the serums of different per-

98. Anderson, J. F., and Frost, W. H.: J. A. M. A. **56**:663, 1911.

99. Flexner, S.: J. A. M. A. **81**:1785, 1923. Zinsser, H., and Tang, F. F.: J. Immunol. **17**:343, 1929. Andrewes, C. H., and Carmichael, E. A.: Lancet **1**: 857, 1930.

100. Aycock, W. L.: J. A. M. A. **87**:75, 1926; Am. J. Hyg. **8**:35, 1928.

101. Aycock, W. L., and Kramer, S. D.: J. Prev. Med. **4**:189, 1930. Kramer, S. D., and Aycock, W. L.: Proc. Soc. Exper. Biol. & Med. **29**:98, 1931.

102. Fairbrother, R. W., and Brown, W. G. S.: Lancet **2**:895, 1930. Shaughnessy, H. J.; Harmon, P. H., and Gordon, F. B.: J. Prev. Med. **4**:463, 1930. Brodie, M.: J. Immunol. **26**:337, 1934; **27**:395, 1934; **28**:1, 1935.

sons. If a single substance is responsible for all of these destructive effects, the immunization theory alone is insufficient to explain its presence in the blood of man.

Some evidence for the nonspecificity of the "antisubstance" in serum has appeared in the literature. Gordon and his co-workers,[103] in an extensive series of papers, which support an earlier point of view of Muir and Browning [104] on the bactericidal activity of blood, have considered that the bactericidal antibody is a nonspecific substance. Differences in the effect of serum on various species of bacteria are thought related not to the presence of an antibody for one and the absence of an antibody for another, but to the relative susceptibility of the several species of bacteria to the single nonspecific antibody of the serum. The work of Mackie and Finkelstein,[105] on the other hand, indicates that the bactericidal antibody is specific for the particular organism acted on. In an effort to explain the results of Gordon, Finkelstein [106] has postulated the existence of a nonspecific factor in suspensions of heated bacterial cultures which inhibits the bactericidal activity of serum and which obscures the specific nature of the absorption of bactericidal substance by bacterial antigens.

Weyer [107] has attempted to show that a single "pan-immune body" in normal human serum neutralizes the two viruses poliomyelitis and herpes. The work of others, however, chiefly Gay and Holden,[108] indicates no correlation between the neutralizing antibodies against these two disease agents.

Strong and I [28] have attempted to relate the trypanocidal with the bactericidal substances of human serum. We found it possible to remove the trypanocidal property simultaneously with the bactericidal activity by absorption of a serum with a mass of bacteria or, less satisfactorily, with a mass of trypanosomes. In a test on a single serum supplied by me, from which both the bactericidal and trypanocidal substances had been absorbed, Jungeblut [109] noted, however, that the poliocidal power was not significantly reduced.[110] Similarly, according to the results of preliminary tests by Strong,[111] such absorption fails to remove

103. Gordon, J.: J. Path. & Bact. **37**:367, 1933. Gordon, J., and Carter, H. S.: ibid. **35**:549, 1932.

104. Muir, R., and Browning, C. H.: J. Path. & Bact. **13**:76, 1909.

105. Mackie, T. J., and Finkelstein, M. H.: J. Hyg. **32**:1, 1932.

106. Finkelstein, M. H.: J. Path. & Bact. **33**:359, 1933.

107. Weyer, E. R.: Proc. Soc. Exper. Biol. & Med. **30**:309, 1932.

108. Gay, F. P., and Holden, M.: J. Infect. Dis. **53**:287, 1933.

109. Jungeblut, C. W.: Personal communication.

110. It is perhaps significant to note in this connection that Jungeblut has been able to absorb the poliocidal substance from human serum by contact in vitro with a suspension of red cells (J. Immunol. **27**:17, 1934).

111. Strong, P. S.: Personal communication.

the activity of the serum against the virus of herpes. This seems to indicate that the trypanocidal substance is more closely related to the bactericidal than to the virucidal substances.

A different relationship, quite as deserving of consideration, but especially difficult to explain on the basis of specific immunization, can also be pointed out: The trypanocidal and virucidal activities are exhibited almost exclusively by the serum of man, whereas the bactericidal property is manifested by the blood of many species of animals. It is generally stated that the serums of many normal adults fail to show activity against the viruses of herpes or poliomyelitis, while the trypanocidal substance is always present in healthy persons after the first few months of life. However, these differences are probably only quantitative, the failure to find some trace of virucidal activity in so-called negative serums being due to insufficient delicacy of the means of testing. From this point of view, the trypanocidal activity seems more closely related with the virucidal than with the bactericidal activity of the serum.

Because of the confusion in which these parallelisms and contrasts remain, it is impossible as yet to draw conclusions on the identity or difference between that substance responsible for the trypanocidal activity of human serum and those responsible for the bactericidal and virucidal actions. The question is certainly one of fundamental significance in immunology, but its solution must await the fruits of the extended investigation which surely the future will bring.

SUMMARY

The normal serum of man will destroy the trypanosomes pathogenic for animals (e. g., T. Brucei, T. equiperdum, T. equinum, etc.) either in the test tube or in the body of rodents infected with these parasites. Human serum does not affect the trypanosomes pathogenic for man (T. gambiense, T. rhodesiense or T. Cruzi), the common trypanosome of rats (T. Lewisi) or a trypanosome of newts (T. diemyctyli). One of the human trypanosomes, T. rhodesiense, is distinctive in that it becomes susceptible to the action of human serum after it has been passed successively through mice. The serum of no other animals, excepting certain monkeys, manifests trypanocidal activity. The serum of some monkeys, however, e. g., that of the baboon, destroys not only the trypanosomes pathogenic for the lower animals but, as well, those infective for man.

The trypanocidal substance of human serum is found in the globulin fraction of the serum. It is thermolabile, being destroyed wholly when the serum is heated at 64 C. for an hour, and being reduced rapidly when the serum is let stand at room temperature. The trypanocidal

substance passes readily through Berkefeld filters and with diminished intensity through collodion ultrafilters. It is removed from a serum by absorption with trypanosomes or bacteria (the typhoid bacillus; Proteus). The substance exhibits its activity independently of all the known components of alexin. The essential substance in the human serum which brings about the trypanocidal effect is antigenic, and a specific antitrypanocidal antibody develops in rabbits repeatedly treated with an active serum.

The trypanocidal substance probably originates in the normally functioning healthy liver. It is found in the blood serum and in serous exudates. The cerebrospinal fluid and the urine are without trypanocidal power. The substance appears in infants at a very early age and may be present at birth. It is probably elaborated within the body of the young child, since it is found neither to pass the placenta nor to occur in human milk. The trypanocidal power is enhanced in women late in the period of gestation and is maintained at a high level for some time after delivery.

It seems unlikely that the action of the trypanocidal substance is that of an opsonin or an agglutinin, and the property is manifested wholly without the intervention of alexin. Some investigators have felt it acts essentially as a chemotherapeutic substance. Susceptible strains of trypanosomes become resistant or fast to human serum after repeated exposure to the serum in a manner comparable to that in which they become resistant or fast to drugs.

Since human serum affects only those species of trypanosomes which are pathogenic for animals and which are noninfective for man, and is without effect on the trypanosomes which are infective for man, it is by some believed that the trypanocidal action of the serum is responsible for man's immunity to the animal pathogens. It is known, however, that strains of animal trypanosomes which have been rendered serum-fast still are noninfective for man, and that strains of human trypanosomes (T. rhodesiense) which become susceptible to human serum after repeated passage through animals retain their infectivity for man. Furthermore, the serum-resistant parasite T. Lewisi, which is widespread among rats, is apparently unable to infect man. The serum of patients with trypanosomiasis is as active in trypanocidal power as that of normal persons. It appears, therefore, that the immunity of man to the animal trypanosomes depends on factors other than the trypanocidal activity of the serum.

The trypanocidal activity of human serum is sharply reduced in diseases which cause extensive destruction of the parenchyma of the liver. Less conclusive evidence of alteration in the potency of the substance has been offered in other infectious diseases (e. g., tuberculosis and syphilis) and in those attributable to deficiencies in accessory food fac-

tors. No significant reduction is observed in human trypanosomiasis. It seems possible, from the observations of some workers, that the carrier condition in hemophilia can be identified by the absence of the trypanocidal substance from the serum.

It appears not unlikely that trypanosomes are but one form of infectious agent on which a single destructive entity in human serum acts. The fact that both the trypanocidal and bactericidal substances are removed from the serum by absorption with either trypanosomes or bacteria points toward a close similarity between the trypanocidal and bactericidal powers. The trypanocidal activity, however, differs from the bactericidal property and resembles the virus-neutralizing function of human serum in being limited to the serum of man and a few closely related primates and in occurring without the presence of alexin. If further study indicates that these effects are all manifestations of a single entity of the serum or, what seems more likely, that they arise in analogous manners, support for the assumption that these "antisubstances" arise by immunization through contact with the specific antigen is difficult to maintain, since at no time in life does the average person suffer subclinical or abortive infection with trypanosomes.

Notes and News

University News, Promotions, Resignations, Appointments. Deaths, etc.—George B. McGrath, medical examiner of Suffolk County (Boston) since 1907 and professor of legal medicine in the Harvard University Medical School since 1931, has resigned because of ill health. He will be succeeded as examiner by William J. Brickley, associate examiner.

Edwin Raymond LeCount, professor of pathology and chairman of the department of pathology at Rush Medical College, died on Aug. 23, 1935, at the age of 67.

Max Pinner, Desert Sanatorium, Tucson, Ariz., has been appointed principal diagnostic pathologist to the hospitals for tuberculosis of the state department of health, New York.

Gustav Hauser, professor emeritus of general pathology and morbid anatomy in the University of Erlangen, has died at the age of 79.

Bela Halpert, assistant professor of pathology and surgery in Yale University, has been appointed head of the division of pathology and associate director of laboratories in the Jewish Hospital of Brooklyn.

John I. Fanz, professor of pathology in Temple University, has died at the age of 44.

Neil McLeod, instructor of pathology in the University of Pennsylvania, has died of injuries received in an automobile accident.

Lydia Rabinovitch Kemper, who was the only woman co-worker of Koch and for many years director of the bacteriologic institute of the Moabite (now Koch) Hospital, Berlin, died on August 5 at the age of 64. She was instructor in bacteriology in the Woman's Medical College in Philadelphia in 1896-1899.

Alexander C. Abbott, professor emeritus of hygiene and bacteriology in the University of Pennsylvania, has died at the age of 75.

Charles Norris, chief medical examiner of New York City since 1918, died on Sept. 11, 1935, at the age of 67.

Awards.—The Sedgwick Memorial Medal of the American Public Health Association has been awarded to Haven Emerson, professor of public health administration in Columbia University.

The medical faculty of the University of Bern, Switzerland, has awarded a prize of 1,000 Swiss francs to Leslie T. Webster, of the Rockefeller Institute for Medical Research, for his investigations in the field of encephalitis.

Fund for the Study of Dementia Praecox.—According to *Science* a fund of $40,000 has been donated by the Supreme Council, Scottish Rite Masons, for the study of dementia praecox. The research program will be under the direction of the National Committee for Mental Hygiene, New York City. The field representative is Nolan D. C. Lewis, formerly director of laboratories of St. Elizabeth's Hospital, Washington, D. C., now director of clinical pathologic research of the Neurological Institute and professor of neuropathology in Columbia University.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

EXPERIMENTAL PRODUCTION OF ANKYLOPOIETIC ARTHRITIS. K. SONNENBERG, Virchows Arch. f. path. Anat. **293**:724, 1934.

By repeatedly injecting horse serum into knee joints of rabbits sensitized to the serum Klinge had been able to produce arthritis deformans. The object of Sonnenberg's work was to determine whether a similar procedure would lead to ankylosing arthritis if the joint was completely immobilized. In each of a series of rabbits a knee joint was immobilized by a surgical procedure. After recovery each animal was sensitized by a single injection of 4 cc. of horse serum. Six weeks later a series of from four to six injections of from 1 to 2 cc. of the sensitizing serum directly into the immobilized joint was begun. The injections were spaced at intervals of from eight to fourteen days. The animals were killed at varying intervals and the joints examined histologically. The local allergic reaction led to inflammatory union of the synovia with the joint cartilages. Ultimately there resulted fibrous and cartilaginous ankylosis of the joint. From his own and Klinge's work Sonnenberg concludes that allergic inflammation of a mobile hyperergic joint leads to arthritis deformans; that similar inflammation of a completely immobilized joint leads to ankylopoietic arthritis; that allergic inflammation of an incompletely immobilized joint leads to a combination of arthritis deformans and ankylopoietic arthritis, and that immobilization without inflammation never leads to ankylosis.

O. T. SCHULTZ.

PHYSIOLOGY AND PATHOLOGY OF THE CIRCULATION. A. BIER, Virchows Arch. f. path. Anat. **293**:738, 1934.

This is a fifty-six page continuation of an article the first part of which appeared in volume 291 of *Virchows Archiv*. By numerous clinical observations and by discussion of them Bier seeks to establish the correctness of his attraction theory of the circulation. He considers the hemodynamics of the circulatory system inadequate to explain the observations cited. An important factor in the maintenance or the reestablishment of local circulation is the need or "hunger" of tissues and organs for fresh blood, required by nutrition and function and for the removal of waste products. By participation of small vessels and capillaries blood is drawn into the tissue by suction in a quantity greater than can be explained by the circulatory mechanics of the heart and large vessels

O. T. SCHULTZ.

ERYTHROCONTES AND ERYTHROPOIESIS IN PERNICIOUS ANEMIA AND IN THE EMBRYO. F. ZANATY, Virchows Arch. f. path. Anat. **293**:794, 1934.

In 1928 Schilling applied the term "erythrocontes" to minute rodlike bodies seen in the polychromatic megalo-erythrocytes of pernicious anemia. He at first believed these bodies to be parasites belonging to the bartonella group. More recently he has held that they are probably derived from the constituents of polychromatic megalocytes. In an attempt to determine the nature of erythrocontes and their possible specific relationship to pernicious anemia, Zanaty examined the bone marrow and spleen of persons dying of diseases other than blood dyscrasias and the blood of human embryos of the third to the ninth month, the latter because a macrocytic state of the blood is physiologic in the embryo. Erythrocontes were not seen in any of the material studied, and Zanaty therefore concludes that they have no relationship to the polychromatophilia of either normocytic or macrocytic

blood. The failure to discover basophilic stippling of fetal erythrocytes is proof of a fundamental difference between the polychromatophilia of fetal and that of postfetal blood. He does not accept the view that pernicious anemia is characterized by a return to a fetal type of erythropoiesis. The megaloblasts of pernicious anemia are morphologically different from those of fetal blood. Zanaty does not accept Naegeli's view that megaloblasts and macroblasts are distinct types of cells, but holds that they are a single type of cell.

O. T. SCHULTZ.

Pathologic Anatomy

TUBEROUS SCLEROSIS WITH UNUSUAL LESIONS OF THE BONES. JACQUES S. GOTTLIEB and GEORGE R. LAVINE, Arch. Neurol. & Psychiat. **33**:379, 1935.

Clinically tuberous sclerosis is characterized by adenoma sebaceum, convulsive seizures and mental deficiency. Any member of the triad may be absent, and there are always neoplasms in some viscera (kidneys, spleen, retina, lungs). In the present case, that of a woman aged 23, mental symptoms were present—the patient was noisy and destructive. There was a previous history of epilepsy and idiocy. At birth, a reddish-brown patch was present on the right side of the neck. This gradually grew larger, and in addition raised white patches and "reddened nodules" appeared on the face (adenoma sebaceum). Tumors in the retina (phakomas) and spina bifida of the sacrum were also found. One of the retinal tumors exhibited at its periphery numerous fine capillaries dipping into its substance, and periosteal thickening with general osteoporosis of both metatarsal and metacarpal bones and their phalanges was in evidence. Several of the bones of the hands showed areas of marked rarefaction, suggesting cysts. There was osteoporosis of the skull.

G. B. HASSIN.

FAT TISSUE IN THYROID ADENOMA. F. BRENNER, Centralbl. f. allg. Path. u. path. Anat. **62**:113, 1935.

In a portion of thyroid gland, 10 by 8 by 3 cm., five adenomas ranging in size to that of a walnut were encountered. Microscopically these were of the microfollicular type with a partially hyalinized stroma. Fat tissue in masses up to 1.5 mm. in diameter occurred near the periphery of the adenomas and were separated from the connective tissue capsule by a band of parenchyma 0.5 mm. wide. In the larger fat masses the cells lay next to one another and resembled typical fat tissue. At the borders of such fields single fat cells occurred between the small alveoli of the adenoma. The alveoli exhibited no retrogression changes. Replacement of gland tissue by fat is well known in such places as the salivary glands and pancreas, where the fat cells originate in the connective tissue cells of the organ. By analogy Brenner believes that in this instance metaplastic changes had occurred in the adenomas, the parenchyma of which was retrogressing.

GEORGE RUKSTINAT.

AN UNUSUAL ANOMALOUS BAND OF THE LEFT AURICLE. K. SCHWEIKART, Centralbl. f. allg. Path. u. path. Anat. **62**:114, 1935.

Anomalous tendinous bands of the left auricle occur seldom as compared with such bands of the right auricle but have a certain uniformity. This consists in the attachment of one end to the valvula foraminis ovalis and of the other to a mitral leaflet or to the ventricular endocardium. In the present case the attachment to the mitral leaflet only was demonstrable. The free end was, however, bifid and matched depressions in the valvula foraminis ovalis. Presumably detachment was effected during marked cardiac dilatation in a woman who suffered from nephritis and mitral stenosis with insufficiency. Death was due to uremia.

GEORGE RUKSTINAT.

PATHOLOGIC AND ANATOMIC CHANGES IN SO-CALLED LEPROSY OF RATS. R. JAFFÉ and G. KAHLAU, Frankfurt. Ztschr. f. Path. **46**:218, 1933.

Stefansky in 1903 described a disease in rats which closely resembled human leprosy. Jaffé and Kahlau examined the organs of sixty rats in which this disease had been produced experimentally. As controls, organs of guinea-pigs which had received injections of leprosy bacilli isolated from human lesions were examined. In the rat the disease was characterized by nodules in the skin and lymph nodes. Microscopically they consisted of granulation tissue with large cells which contained much cytoplasm and also a number of acid-fast bacilli. These cells were referred to as leprosy cells. Bacilli were also found in the Kupffer cells of the liver, in the spleen and in the cells of the reticulo-endothelial system in other organs. These cells were thought to be the origin of the leprosy cells. Later, round cells and giant cells of the Langerhans type were found in addition to the leprosy cells. Whereas in the rat the testes were never involved, the testes in man very often showed the characteristic granulation tissue. In man the leprosy cells showed many vacuoles while these were not present in those of the rat. The characteristic arrangement of the bacilli within the leprosy cell so often seen in man was not found in the rat. The authors conclude that the changes in the organs in man closely resemble but are not identical with those in the rat. OTTO SAPHIR.

MALFORMATION OF THE LOWER EXTREMITIES (SIRENOMELUS). E. HEITS, Frankfurt. Ztschr. f. Path. **46**:241, 1933.

Heits examined two cases of this anomaly. In the first the sacrum and coccyx were missing and only four lumbar vertebrae were recognizable. Both iliac bones were horizontally placed and were adherent to each other. The ischiatic and iliac bones were also firmly adherent to each other. The pubic bones showed no changes. There was a rudimentary tibial bone and two patellae. In the second instance the changes in the lower extremities were similar to those in the first instance. In addition there were revealed thin soft parietal bones and hydrocephalus. Also the right thumb was absent. Heits concludes that the sirenoformation results from a primary arrest of the development of the caudal segment of the body occurring probably in the first half of the third week of the embryonal development. The cause of this malformation is apparently exogenous, for various malformations of other organs may be present. OTTO SAPHIR.

MARKED DEFORMITY OF THE BRAIN FROM MENINGEAL CYST. D. SCHRANZ, Frankfurt. Ztschr. f. Path. **46**:252, 1933.

In a 50 year old man who committed suicide a cyst was found in the left sylvian fissure. The cyst measured 8.5 by 9 cm. in diameter and extended from the orbital portion of the inferior frontal gyrus to the lower portion of the central gyrus and reached the temporal gyri. The pia mater over the cyst was markedly thickened and milky white. The brain was greatly compressed but did not reveal any other gross or histologic change. The history showed absence of trauma but brought out that he always had been left-handed. Schranz does not believe that the cyst was congenital, but thinks it developed later in life, possibly as a result of an old healed circumscribed meningitis. OTTO SAPHIR.

BRAIN CHANGES IN LEUKEMIA. W. HAMBURGER, Frankfurt. Ztschr. f. Path. **46**:257, 1933.

Hamburger examined four cases of myeloid leukemia and one of lymphatic leukemia. The hemorrhages in the brain seen in leukemia are grossly different from those due to other causes. The former are firmer and are distinctly yellowish brown. They are often diffusely present throughout the white substance, are often small and resemble those seen in hemorrhagic encephalitis. The capillaries are dilated and often filled to capacity with white blood corpuscles. This condition is

spoken of as white stasis. The hemorrhages are the result of diapedesis of the blood corpuscles. Since often most of the cells in the vessels are white blood cells, the hemorrhages may give the erroneous impression of leukemic infiltration.

OTTO SAPHIR.

MUCUS-CONTAINING STONES IN THE APPENDIX. J. VON SOÓS, Frankfurt. Ztschr. f. Path. **46**:286, 1933.

An appendix containing 134 round stones covered with and also containing mucus was removed at autopsy from a 69 year old man. The appendix was 8 cm. long and spindle-shaped, and its orifice was markedly narrowed. The stones measured from 1 to 3 mm. in diameter. In the center of each was a yellow opaque granule. On chemical examination it was shown that the calculi consisted of an albuminous material, phosphates and calcium carbonates. Von Soós believes that the occurrence of mucus within the stone is a secondary process. The patient had been a stone cutter and apparently had swallowed the particles of stone which formed the nuclei of the calculi.

OTTO SAPHIR.

NECROSIS OF THE PANCREAS. W. MÄNZ, Frankfurt. Ztschr. f. Path. **46**:295, 1933.

Twenty-five instances of acute and subacute necrosis of the pancreas are reported. In the majority of these cases gallstones were found. In some instances fat necrosis and the necrosis of the pancreas were the result of carcinoma which caused either primary pressure necrosis of the pancreatic parenchyma or narrowing of the ducts. Occasionally the disease was brought about by the presence of ascarides within the ducts with resulting severe inflammation. Rarely a primary chronic inflammation of the duct was thought to have caused the necrosis of the pancreas. Very occasionally histologically demonstrable disease of the pancreatic arteries caused the necrosis of the pancreas. Mänz does not believe that primary necrosis of the pancreas may occur in the absence of inflammatory changes as was thought by Baló (*Virchows Arch. f. path. Anat.* **259**:733, 1926).

AUTHOR'S SUMMARY.

AMYLOID CONTRACTED KIDNEYS. H. WILLER, Frankfurt. Ztschr. f. Path. **46**:306, 1933.

Willer reports three cases of uncomplicated amyloid infiltration of both kidneys which had caused contraction of these organs. The ages of the patients were 31, 39 and 42, respectively. They all had general edema and much urinary albumin. The nonprotein nitrogen of the blood was determined for two patients and amounted to 32 mg. and 117 mg. per hundred cubic centimeters, respectively. The arterial blood pressure was 125 systolic and 75 diastolic in the first, 120 systolic and 75 diastolic in the second and 120 systolic and 80 diastolic in the third patient. The eyegrounds were normal. The autopsies revealed neither arteriosclerosis nor cardiac hypertrophy. The kidneys grossly were smaller than normal. Their surfaces were grayish white to yellow with irregularly outlined retracted areas. The cut surfaces were glossy brownish, and the cortex was markedly reduced in size. Histologically almost every glomerulus showed changes. Most of the glomeruli were enlarged; occasionally one was contracted. They were poor in nuclei and almost completely replaced by a homogeneous substance which gave the characteristic amyloid staining reactions. Bowman's capsules were thickened and contained much newly formed connective tissue, which occasionally extended into the loops of the glomeruli. The tubuli revealed changes which were characteristic of lipoid nephrosis. The interstitial tissue showed many scars and masses of amyloid. The lumens of the smaller arteries and particularly of those of the vasa afferentia were narrowed by rings of amyloid. Also a fourth case is reported. The patient was 77 years old. The arterial blood pressure was 140 systolic and 90 diastolic, and there also was marked albuminuria. The heart was not enlarged. The kidneys were similar

to those seen in the first three instances. Rats in which amyloid contracted kidneys were produced experimentally did not reveal hypertrophy of the heart.

Willer stresses the point that even though in these four instances both kidneys were severely diseased the patients did not show hypertension and the hearts were not hypertrophic at autopsy. This, he believes, speaks against the assumption that renal lesions and arterial changes are the primary factors in hypertension.

OTTO SAPHIR.

CONTRACTED SOLITARY KIDNEY. H. WILLER, Frankfurt. Ztschr. f. Path. **46**:321, 1933.

Willer reports two cases. In one there was agenesia of the left kidney and left ureter and chronic glomerulonephritis in the right kidney. The patient died of uremia. The arterial blood pressure was 190 systolic and 110 diastolic declining to 155 systolic and 95 diastolic. The nonprotein nitrogen content of the blood was 79 mg. per hundred cubic centimeters and increased to 112 mg. There was albumin in the urine. The second patient had an arterial blood pressure of 185 systolic and 100 diastolic. The urine contained much albumin. At autopsy only a minute portion of renal tissue was found in place of the left kidney. The right was an arteriolosclerotic contracted kidney. The minute portion of the left kidney revealed the presence of only a few collecting tubules; neither convoluted tubules nor glomeruli were found. Willer stresses the fact that in spite of the marked decrease of the parenchyma of the kidney in each instance the hypertrophy of the left cardiac ventricle in each instance was relatively slight. He also maintains that the increase in systolic pressure is less than one would expect considering the severe loss of renal tissue. This indicates to him that there is no ratio between parenchymal changes in the kidney, on one hand, and hypertension and hypertrophy of the left cardiac ventricle, on the other.

OTTO SAPHIR.

HISTOLOGY OF THE EXTRAMURAL CARDIAC NERVOUS SYSTEM IN CASES OF SUDDEN DEATH. M. I. AWDEJEW ET AL., Virchows Arch. f. path. Anat. **293**:351, 1934.

The anatomic substrate of suddenly fatal cardiac failure was sought first in the myocardium. Later the intrinsic conduction system of the heart became the subject of investigation. In the study here reported attention was directed to the extramural cardiac nervous system: the vagus nerve and its ganglion and the cervical sympathetic nerve and the superior cervical and stellate ganglions. The material, which was investigated chiefly by means of a modified Bielschowsky method, was derived from thirty-one cases of sudden death which had been subjected to medicolegal necropsy. Twenty-four were cases of coronary arteriosclerosis with the usual concomitants of this condition, namely, thrombosis, myomalacia, myofibrosis and cardiac aneurysm with rupture. Four were cases of syphilitic mesaortitis with aneurysm but without rupture; two were cases of marked cardiosclerosis without arteriosclerosis, and one was a case of fatal epilepsy in which the heart and vessels were normal. The ages of the subjects varied from 27 to 85 years. Material from thirteen controls varying in age from 5 to 73 years was also studied. In coronary arteriosclerosis the vagus and sympathetic ganglions revealed a condition of fibrotic shrinkage. This is held to be, not the cause of the arteriosclerosis, but the result of the latter. The alteration to which great importance is attached is globular swelling of the processes of the nerve cells and proliferation of axis-cylinders with the formation of networks of fibrils about and between the groups of ganglion cells. With these changes in the ganglion were associated enlargement, nodular swelling and irregularity in the course of nerve fibrils in the trunk of the vagus. Similar alterations were not observed in the sympathetic ganglions or the sympathetic trunk. The histologic changes observed are held to be the result of a state of hyperirritability of the vagus and its ganglion. To this state are ascribed the attacks of angina pectoris which subjects with coronary sclerosis were known to have had, as well as the sudden stoppage of the heart.

O. T. SCHULTZ.

CHANGES IN THE TEETH IN TUMORS OF THE JAW. M. N. ZAJEWLOSCHIN and S. I. LIBIN, Virchows Arch. f. path. Anat. **293**:364, 1934.

A microscopic study of the teeth was made in fifty-six cases of neoplasm of the jaw. In most of the cases the growth was a primary tumor of the jaw; in some it was a tumor of a contiguous structure that involved the jaw by continuity; in two it was metastatic. There were observed degenerative, inflammatory and necrobiotic changes in the pulp and pericementum and resorptive and appositional alterations in the hard structures of the teeth. Since such changes were noted in teeth at some distance from the tumor they are ascribed, not to direct action of the tumor, but to chemical changes brought about by the tumor. In a rather surprising percentage of the cases of malignant tumor tumor cells were seen in the pulp.

O. T. SCHULTZ.

CHANGES IN THE STERNOCLAVICULAR JOINT IN RELATION TO AGE. P. LANGEN, Virchows Arch. f. path. Anat. **293**:381, 1935.

Both sternoclavicular joints were subjected to microscopic study in 200 cases. The subjects ranged in age from prematurity to 85 years. The histologic character of the joint at various periods of life is described. At 25 to 30 years of age degenerative and regressive changes in the cartilage make their appearance and are followed by increased vascularity and reorganization of the underlying bone. By the age of 40 years these changes are evident in every person and have resulted in a state which Langen terms arthritis deformans. The changes described, and by inference those of arthritis deformans, lie at the boundary between the physiologic and the pathologic.

O. T. SCHULTZ.

RESISTANCE OF THE GANGLION CELLS IN CERTAIN DISEASES OF THE NERVOUS SYSTEM. HANS-JOACHIM SCHERER, Virchows Arch. f. path. Anat. **293**:429, 1934.

Parenchymatous cells in general are more susceptible to harmful agents than supporting cells. The same doctrine is accepted for the nervous system, the ganglion cells of which are most vulnerable. These cells, however, were found to be highly resistant to infiltrating glioblastoma of the brain, to multiple sclerosis and to Wilson's disease in the involved areas of the brain. The vulnerability of the ganglion cells is most evident in vascular disturbances of the brain.

O. T. SCHULTZ.

FATAL PERICARDIAL HEMORRHAGE DUE TO RUPTURE OF A CORONARY ARTERY. JANINA KOWALCZYKOVA, Virchows Arch. f. path. Anat. **293**:464, 1934.

Necropsy of an 80 year old man with senile arteriosclerotic gangrene of the foot who died suddenly revealed a massive hemorrhage into the pericardial cavity. The hemorrhage had come from a ruptured sclerotic and atheromatous subepicardial branch of the left coronary artery and was not the result of myocardial necrosis. The author could find no record of a similar spontaneous nontraumatic rupture of a diseased coronary artery.

O. T. SCHULTZ.

Immunology

ANAPHYLACTOGENIC PROPERTIES OF MILK. B. RATNER and H. L. GRUEHL, Am. J. Dis. Child. **49**:287, 1935.

The proteins isolated from raw milk are chemically and biologically distinct. There are no antigenic changes as a result of drying, acidification or pasteurization. Dried, acidified, superheated or evaporated milk shows no loss of the antigenic properties of the casein fraction. As a sensitizing agent given by injection

milk that has been evaporated, freshly boiled for several hours or superheated shows practically no loss of the antigenic character of the lactalbumin. As a shock agent by injection evaporated or superheated milk shows an unmistakable loss of the antigenic properties of the whey fraction. This is more marked with evaporated than with superheated milk (smaco 303). Evaporated, freshly boiled and acidified evaporated milks, when fed by mouth, show a marked reduction in sensitizing ability. The loss of the antigenic properties of heated milks is presumably due to coagulation of the whey proteins, for which there is some evidence of reversibility. The further reduction in the antigenic properties of heated milks when fed by mouth is due to the fact that coagulation delays the passage of proteins through the gastro-intestinal tract, thus making for more complete digestion and diminishing the probability of the absorption of native antigens through the intestinal wall. In view of the experimental and clinical observations recorded here it appears that evaporated milk is the modification of greatest value for the person who is sensitive to milk.

From the Authors' Summary.

Anaphylactogenic Properties of Malted Sugars and Corn Syrup. B. Ratner and H. L. Gruehl, Am. J. Dis. Child. **49**:307, 1935.

Malt extracts and the barley malt from which they are derived are highly anaphylactogenic and animals sensitized to them react specifically to the original protein constituent, hordein, present in barley. Persons who are sensitive to barley may show symptoms after the ingestion of malt extracts and malt brews. Corn syrups and pure dextrimaltose sugars are nonanaphylactogenic. It is improbable that the highly purified and refined sugars have anaphylactogenic properties. The addition of wheat germ or dried milk to nonanaphylactogenic preparations of dextrimaltose converts them into substances which are definitely anaphylactogenic. Persons sensitive to wheat, yeast or milk may have allergic exacerbations from the use of such compound preparations of dextrimaltose. Those who cannot tolerate honey may be sensitive to the specific protein elements of the nectar from which it is derived, for example, buckwheat. Allergy to carbohydrate foods cannot therefore be attributed to the carbohydrates per se but must be ascribed to the protein constituents which are added to certain compound carbohydrate food preparations. The experiments reported make it apparent that pure dextrimaltose, corn syrup and crystalline sugars play no rôle in allergy.

From the Authors' Conclusions.

Effect of Acute Diseases on the Reaction of the Skin to Tuberculin. A. G. Mitchell et al., Am. J. Dis. Child. **49**:695, 1935.

A depression of allergy to tuberculin exists during the acute stages of scarlet fever, and probably of measles, which disappears during the convalescent stage. This is shown: (1) by significant differences in the occurrence of positive reactions during the acute and convalescent stages; (2) by the depression of allergy in the acute stage in persons known to give positive reactions; (3) by a delay in the time of the appearance of the reaction during the acute stage, and (4) by the smaller size of the reaction in the acute stage as compared with that in the convalescent stage. From a practical clinical standpoint it follows that failure to elicit a skin response to tuberculin during the acute stage of scarlet fever, and probably of measles, cannot be accepted as evidence that the person does not normally give a positive reaction. No statistically significant difference can be demonstrated between the rates of positive reaction to tuberculin during the acute and convalescent stages of diphtheria. This is the more conclusive since stable rates of reaction were obtained for the acute and the convalescent stage of the disease.

From the Authors' Summary.

IMMUNITY IN EPIZOOTIC FOX ENCEPHALITIS. R. G. GREEN et al., Am. J. Hyg. **21**:366, 1935.

Recovery from fox encephalitis is accompanied by an acquired permanent immunity, which appears to depend on the presence of an antivirus in the blood stream. The development of the acquired immunity evidently requires several weeks in the most susceptible animals. As fatalities are confined almost entirely to the first week of the disease, acquired immunity probably plays little rôle in individual recovery. It seems that the degree of natural immunity at the onset of the disease determines recovery from this infection. An active antivirus can be developed in serum by hyperimmunization. A maximum antivirus content is obtained in serum only after more than a year of weekly injections. Such a serum shows its activity under experimental conditions only when mixed with the virus before injection. Delayed infection occurs approximately thirty days after the injection of the serum-virus mixture. The delayed infection is marked by the acute symptoms and the presence of the specific inclusions typical of the natural disease. Delayed infection may be prevented by a second injection of serum three weeks after the injection of the serum-virus mixture. Foxes surviving the injection of the serum-virus mixture and aided by a second injection of serum are generally immune to the disease, but about 8 per cent are still susceptible to experimental infection six months later.

FROM THE AUTHORS' SUMMARY.

ALLERGIC REACTIONS OF ACTINOMYCETES. D. R. MATHIESON et al., Am. J. Hyg. **21**:405, 1935.

Infection and immunization with acid-fast actinomycetes tend to produce an allergic sensitization in experimental animals. No cross-sensitization to tuberculin could be demonstrated. Continued immunization leads to desensitization. Normal persons give more frequent and more marked skin reactions to Actinomyces bovis than do actinomycotic patients. Whereas single injections of A. bovis rarely produce infection, repeated inoculations usually do. This is in agreement with the findings of Nakayama, who first suggested that allergic sensitization is a factor in the etiology of actinomycosis. No sensitization demonstrable by skin tests could be induced in rabbits inoculated with saprophytic aerobic actinomycetes. Representative actinomycetes do not elicit reactions similar to those described by Schwartzman for meningococci and other bacteria.

FROM THE AUTHORS' SUMMARY.

IMMUNIZATION WITH FORMOLIZED TISSUE CULTURES OF TYPHUS RICKETTSIA. I. J. KLIGLER and M. ASCHNER, Brit. J. Exper. Path. **15**:337, 1934.

Data are presented showing that it is possible to immunize animals with emulsified tissue cultures of Rickettsia sterilized with dilute solution of formaldehyde. Cultures of the European and Mediterranean rat virus were used, and in each instance three injections, equivalent to about one sixth of a guinea-pig's tunica albuginea testis, proved sufficient to induce an effective immunity. Old as well as fresh cultures and freshly formaldehydized suspensions as well as old ones are equally effective for immunization. The failure to induce immunity with dead Rickettsia in infected tissues appears to be due to the insufficient amount of antigen present in the infected tissues. It is suggested that this is the reason for a similar failure with viruses, and that vaccines made from virus cultures may offer a solution to the problem of active immunization with dead virus.

FROM THE AUTHORS' CONCLUSIONS.

THE PROPERTIES OF THE VI ANTIGEN OF THE TYPHOID BACILLUS. A. FELIX, S. S. BHATNAGAR and R. M. PITT, Brit. J. Exper. Path. **15**:346, 1934.

The Vi antigen of the typhoid bacillus can be demonstrated by the inagglutinability of the living organisms by pure O serum or by their agglutinability with

pure Vi serum. The two methods give equally reliable results. The development of Vi antigen is suppressed when virulent strains of the typhoid bacillus are grown at temperatures between 20 and 25 C. and between 40 and 44.5 C. The application of this technic to similar studies of other bacterial species is suggested. The resistance of the Vi antigen to heat is described as it is reflected by agglutination and absorption tests and by the formation of antibodies in the rabbit. Saline extracts of cultures of virulent strains of the typhoid bacillus contain Vi antigen, precipitable by pure Vi antiserum. The use of formaldehydized extracts is suggested for the preparation of relatively potent Vi antiserum.

From the Authors' Summary.

Antigenic Differences Between Related Bacterial Strains: A Criticism of the Mosaic Hypothesis. F. M. Burnet, Brit. J. Exper. Path. **15**:354, 1934.

An extract of the Flexner dysentery bacillus is composed of immunologically similar molecules of antigen and not of the mixture of antigens postulated in the mosaic theory of bacterial antigenic structure.

From the Author's Conclusions.

The Immunological Relationship of Pseudorabies (Infectious Bulbar Paralysis, Mad Itch). A. B. Sabin, Brit. J. Exper. Path. **15**:372, 1935.

Pseudorabies bears no immunologic relationship to rabies. Pseudorabies virus possesses many properties in common with the virus of herpes simplex but is easily differentiated from it by active and passive immunity tests. Certain hyperimmune antiherpes serums protected guinea-pigs from minimal but constantly infective doses of the virus of pseudorabies. Four of fourteen herpes-immunized guinea-pigs resisted a small but definitely infective dose of pseudorabies virus. A potent antipseudorabies serum had no effect on herpes. A partial immunologic relationship between pseudorabies and herpes is considered possible. A generic relationship is suggested for pseudorabies virus, the B virus and the virus of herpes simplex.

From the Author's Summary.

The Flocculable Substance of Vaccinia. M. H. Salaman, Brit. J. Exper. Path. **15**:381, 1934.

Seitz filtrates of suspensions of the crusts from vaccinial rabbit's skin possess some antigenic power. This power can be definitely enhanced by adsorption of the antigen on particles of collodion. A heat-stable flocculating substance may be prepared from the crusts of vaccinial rabbit's skin similar in properties to that obtained by Wilson Smith from vaccinial rabbit's testicle.

From the Author's Conclusions.

The Agglutinogens of a Strain of Vaccinia Elementary Bodies. J. Craigie and F. O. Wishart, Brit. J. Exper. Path. **15**:390, 1934.

Agglutinating serum for the elementary bodies of vaccinia, obtained by vaccination of rabbits with or without further inoculation of elementary bodies of the C. L. strain of vaccine virus, contains two agglutinins. The two corresponding agglutinogens differ in their relative stability to heat and other agents. The more labile agglutinogen designated L has its agglutinability and ability to absorb agglutinin impaired or destroyed by exposure to a temperature as low as 56 C. The agglutinogen designated S is stable at temperatures up to 95 C.

From the Authors' Conclusions.

SPECIES IMMUNITY TO PNEUMOCOCCUS. DAVID HARLEY, Brit. J. Exper. Path. **16**:14, 1935.

A solution of pneumococcus type-specific antigen which has been made alkaline and heated to 37 C. and injected into mice produces immunity to the homologous and the heterologous types of living pneumococci alike. This new antigen is believed to be a product of a dissociation of the type-specific antigen by the action of the alkali into an antigenic element and a nonantigenic type-specific material. This free antigenic element is common to the type-specific antigens of all the virulent types of pneumococci; it has been designated "pneumococcic species antigen."

FROM THE AUTHOR'S SUMMARY.

THE STREPTOCOCCAL COMPLEMENT-FIXATION REACTION IN RHEUMATIC DISEASES. A. BECK and F. COSTE, Brit. J. Exper. Path. **16**:20, 1935.

Complement-fixation tests have been made on seventy-nine serums from rheumatic patients and on fifty-three control serums. A number of the former group reacted with streptococcic lipoid antigens and failed to react with lipoid antigens of other bacteria or with tuberculous and syphilitic lipoid antigens. Examinations of the control serums showed that the serums of tuberculous persons and of pregnant women often react with streptococcic lipoid antigens. Therefore in assessing the significance of positive streptococcic reactions tuberculosis and pregnancy must be excluded. The positive reaction with a streptococcic antigen has been observed only in those cases of rheumatic disease in which clinically a connection with streptococcic infection was probable. But even in these cases, and also in cases of acute streptococcic infection (scarlet fever, erysipelas), the percentage of serums with positive reactions is not high (seven of forty-four rheumatic serums). It is believed that the sensitiveness of the test is not yet optimal, and an attempt is being made to increase it. If this can be done it may be possible to determine whether rheumatic disease is of streptococcic or other origin.

FROM THE AUTHORS' SUMMARY.

HISTOLOGY OF LOCAL HYPERSENSITIVITY REACTIONS. R. LAPORTE, Ann. Inst. Pasteur **53**:598, 1934.

The tuberculin reaction in guinea-pigs is compared with other hypersensitive reactions in the skin. "The tuberculin reaction is clearly distinguished from the anaphylactic reaction of the skin. In the latter the phenomena are more rapid and more severe; necrosis attacks the tissue (notably the epidermis) and electively attacks the vascular walls resulting in intense hemorrhages; this necrosis of the vascular walls is found in the deep layers of the skin, a characteristic which is not seen in allergic reactions. The marked edema, the relative paucity of inflammatory cells in the skin, the predominance of polymorphonuclears and the accumulation of eosinophils constitute the other principal differential characteristics of anaphylactic reactions." Comparing reactions to other irritating substances with the tuberculin reaction Laporte found many points in common; "nevertheless, it is unequivocal that the tuberculous animal reacts by more precocious and notably more important influx of monocytes."

M. S. MARSHALL.

THE CYTOLYTIC AND TOXIC PROPERTIES OF STAPHYLOCOCCI. C. GENGOU, Arch. internat. de méd. expér. **9**:413, 1935.

Hemolysins, leukocidins and the necrotizing toxin in staphylococcic filtrates are different manifestations of a single product of bacterial metabolism. Since the filtrability of the toxin changes on cultivation of the bacteria in vitro, chloroform has been used in place of filtration for sterilization of the cultures. The amount of precipitate formed in toxic filtrates on addition of antitoxin is not a true index of the strength of the toxin. The precipitate varies with the kind of medium.

ELIZABETH MCBROOM.

ALLERGIC REACTIONS OF THE KIDNEY. M. MASUGI and Y. SATO, Virchows Arch. f. path. Anat. **293**:615, 1934.

In a previous contribution the authors described renal glomerular changes following the injection of large doses of antikidney serum into rabbits. They considered the changes identical with those of human diffuse glomerulonephritis. These alterations they held to be allergic. In the present work rabbits were sensitized by repeated subcutaneous, intraperitoneal or intravenous injections of horse serum or egg-white. In one series of animals the kidneys were subjected to microscopic examination at various stages of sensitization. In another series the examination was made after the injection into the renal artery of a final activating dose of the foreign protein. In both series the authors observed glomerular changes which they consider identical with the characteristic lesions of human diffuse glomerulonephritis, namely, plasma stasis, fibrinothrombosis and blood stasis in the glomerular vessels. The process involved entire glomerular tufts and the glomeruli throughout the kidney. They conclude that human diffuse glomerulonephritis is an allergic reaction in a hyperergic kidney. In some animals they observed perivascular lesions similar to those of periarteritis nodosa, which, therefore, they also look on as an allergic reaction.

O. T. SCHULTZ.

THE ANAPHYLACTIC REACTION AFTER REMOVAL OF INHIBITORY SUBSTANCES. E. BERGER and W. MUTSAARS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **83**:1, 1934.

The addition of an alcoholic solution of cholesterol to the Ringer solution in which the uterine horn of a passively sensitized guinea-pig was suspended inhibited regularly the specific anaphylactic contraction following the addition of the antigen. The mere replacement of the alcoholic solution with fresh Ringer solution brought about a typical contraction. The alcohol was responsible for the inhibition, and not the cholesterol. The effect of histamine was frequently inhibited by the alcoholic solution, but replacement of the latter by Ringer solution was only exceptionally followed by a contraction.

I. DAVIDSOHN.

THE SPECIFICITY OF ANAPHYLACTIC VASCULAR CONTRACTILITY OF WHITE RATS. K. M. DWOILAZKAJA-BARYSCHEWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **83**:31, 1934.

Segments of arteries were perfused with fluid with and without antigenic substances, and the contraction was measured by the decrease in the number of drops which left the blood vessels. A marked contractility was observed in sensitized white rats, but it was not specific, because heterologous as well as homologous antigens (serums and erythrocytes) were able to produce it. This corroborates the claims of Kritschevski and others that the reaction of the sensitized blood vessels following perfusion of antigens is not identical with the anaphylactic shock.

I. DAVIDSOHN.

DIPHTHERIA ANTITOXIN IN THE BLOOD OF THE POPULATION IN A TOWN OF JAVA. JEANNE VAN DEN HOVEN VAN GENDEREN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **83**:42, 1934.

The blood of 1,641 men, women and children of Bandoeng, Java, was studied by means of Römer's technic. A comparative study of the blood of the umbilical cord and of the venous blood of parturient women confirmed the known fact that the amount of diphtheria antitoxin is about equal in both. The native population and the Chinese adults and children had high titers of antitoxin, somewhat lower in women and girls. The titers were much higher than among the European and mixed population, among whom the women showed a particularly low titer.

I. DAVIDSOHN.

Tumors

BRONCHIOGENIC CANCER COMBINED WITH TUBERCULOSIS OF THE LUNGS. B. M. FRIED, Am. J. Cancer **23**:247, 1935.

From a study of thirteen cases of primary carcinoma of the lung combined with pulmonary tuberculosis it was found that the carcinoma had developed independently from the tuberculous disease in some cases, while in others it was engrafted on an old fibrotic infection. The carcinoma did not originate in the wall of the tuberculous cavity but in the basal cells of the adjacent bronchus from where it reached the cavity, lining its wall in a syncytial manner and growing in masses. "Cancerization" of a cavity is analogous to the so-called "epithelialization" of a cavity observed in cases in which the infection with Koch's bacillus shows a tendency toward healing.

In five of the cases the carcinoma was of the small cell variety; in six, squamous epithelial; in one, keratinizing epidermoid; and in one, adenocarcinoma.

All the patients were men. Three showed tubercle bacilli in the sputum, which is contrary to the general belief that the sputum of patients in whom these two diseases are combined is invariably negative for Koch's bacilli.

The tuberculous lesion found in the lungs of the patients was of the healing fibrotic type and was to all appearances of long standing.

The clinical histories and the necropsy observations of the thirteen patients are given in detail. The article is illustrated with ten photomicrographs and ten roentgenograms.

MYXOSARCOMA. A. A. THIBAUDEAU and L. C. KRESS, Am. J. Cancer **23**:267, 1935.

Myxomatous tissue in a tumor is not an evidence of degeneration. Myxomatous tissue is a derivative of connective tissue and can probably originate from the metaplasia of different types of connective tissue. The more malignant a myxomatous tumor, the more cellular it becomes. Myxosarcoma is clinically highly malignant, responding only occasionally to recognized forms of therapy. In general myxosarcoma is resistant to radiation therapy; in some instances, however, it has responded admirably to this type of treatment. Myxosarcoma is most apt to arise between the ages of 40 and 60 years; it shows no special predilection for either sex. The shorter the clinical duration of the tumor before treatment, the more satisfactory is the response to therapy. Myxosarcoma in cases in which recurrences have occurred does not respond to treatment. Widely disseminated metastases occur late in myxosarcoma. Myxofibroma is readily eradicated by appropriate therapy.

FROM THE AUTHORS' SUMMARY.

TUMOR TRANSPLANTS FROM MICE TO SPLENECTOMIZED RATS. J. HEIMAN, Am. J. Cancer **23**:282, 1935.

Transplants of mouse carcinoma 63 and 11 and of sarcoma 180 and 37 will not grow in adult splenectomized or in adult normal rats. Mouse sarcoma 37 will grow for a short time in young normal and young splenectomized rats, but recession and absorption of the tumor occur promptly, without visibly affecting the animals. The growth energy of a tumor may be diminished during its stay in foreign soil but is regained when the tumor is transferred to its normal habitat. The presence or absence of the spleen in a mouse or a rat does not seem to hinder or accelerate the growth of sarcoma 37. The age of the animal influences the growth of heterotransplants.

EFFECT OF BROMCAPROIC ACID ON RAT SARCOMA 39. W. A. SELLE and M. BODANSKY, Am. J. Cancer **23**:289, 1935.

Bromcaproic acid, despite the fact that it permeates cell membranes more readily than the halogen derivatives of the lower fatty acids, was found to exert

no specific inhibitory effect on the growth of rat sarcoma 39 though given in daily doses equal to one third of the lethal dose over a period of three or four weeks.

From the Authors' Conclusion.

The Influence of Magnesium on the Growth of Carcinoma, Sarcoma and Melanoma. K. Sugiura and S. R. Benedict, Am. J. Cancer **23**:300, 1935.

Transplants of the Flexner-Jobling rat carcinoma in rats fed a diet containing magnesium in the concentration of only 1.8 parts per million, but otherwise adequate, survived more frequently but grew very much more slowly than such transplants in animals receiving a magnesium-normal diet or fed the common diet. At the end of the third week, the weight of the tumor in the rat fed the magnesium-low diet was only about 4 per cent of that in the animal fed the magnesium-normal diet. The rate of tumor growth in the animals to the diet of which a small amount of magnesium in the form of magnesium sulphate had been added (0.0108 per cent magnesium) was decidedly diminished, but this inhibition was not so great as in the animals fed a diet practically free from magnesium. The resistance of the latter animals to tumor regression was definitely increased over that of normally fed animals. When young rats in which cancerous grafts had become well established were placed on a magnesium-low diet (0.00018 per cent magnesium), there was no marked inhibitory effect on the subsequent rate of tumor growth. When magnesium in the form of magnesium sulphate was restored in adequate amount (0.0538 per cent magnesium) to the magnesium-low diet (0.00018 per cent magnesium) of tumor-bearing animals the tumor nodules grew very rapidly and attained normal size in seven days. A magnesium-high diet (0.1775 per cent magnesium) had a slight but distinct accelerating effect on the growth of the Flexner-Jobling rat carcinoma. However, there was a slight increase in the number of tumor regressions in rats on this diet. Prolonged feeding of the magnesium-high diet did not have any effect in checking the growth of firmly established Flexner-Jobling rat carcinoma, mouse sarcoma 180 and Passey mouse melanoma. The results cast doubt on the value of magnesium in the treatment of human neoplasms. They also show that a neoplasm, like normal tissue, requires a definite amount of magnesium for its growth.

From the Authors' Summary.

On the Physiological Validity of Enzyme (Amylase) Determinations in Tumor Tissue. F. H. Scharles, P. D. Robb and W. T. Salter, Am. J. Cancer **23**:322, 1935.

A method has been previously described whereby the amylolytic effect of a tumor extract can be expressed as a logarithmic function of the concentration of the extract. Assays are presented of mixtures of varying proportions of two sarcoma extracts. In each case the value obtained agrees with the sum of the theoretically calculated partial activities of the respective components. Because the partial activities are directly additive, it is suggested that the method determines purely the activity of enzyme, and not the effect of accompanying accelerators or inhibitors. This fact substantiates the contention that appropriate determinations of the enzyme activities in tissues have a fundamental physiologic meaning.

From the Authors' Summary.

Neurocytoma Derived from a Ganglioneuroma of the Hypogastric Plexus. Samuel J. Hoffman, Am. J. Dis. Child. **49**:135, 1935.

This case is interesting because it shows the nonmalignant form of neurocytoma in the form of a ganglioneuroma and also the true neurocytoma in the same section. It shows an unusual form of metastasis to many bones in which the periosteum was uniformly thickened.

From the Author's Conclusions.

A Malignant Hemangioma of the Lung with Multiple Metastases. E. M. Hall, Am. J. Path. **11**:343, 1935.

A case of malignant metastasizing hemangioma, of which there are less than a dozen true cases recorded in the whole of medical literature, is reported. The largest tumor was found in the right lung, and death was due to hemorrhage into the right pleural cavity. True metastases consisting of both cavernous and malignant cellular areas were found in the lungs, pleurae, retroperitoneal lymph nodes and liver. The type cell was the endothelial cell, which formed blood-vascular spaces in all the tumor nodules. The cells varied from practically normal-appearing ones lining the cavernous spaces to extremely large atypical cells that almost filled the blood spaces in the more cellular areas. In the latter the growth was rapid and apparently highly malignant.

From the Author's Summary.

A Ganglioneuroma in the Neck of a Child. J. MacFarland and S. W. Sappington, Am. J. Path. **11**:429, 1935.

The case described is that of a well characterized ganglioneuroma. In this tumor, however, nerve cells of all stages of development from neuroblasts to ganglion cells occurred, and among them was a stroma made up of Schwann cells and nerve fibers. It occurred in the neck of a little girl, and the case seems to be the twelfth of its kind to be placed on record. Three years after operative removal the patient is living, with no return of the tumor and no metastases. Appended to the article are 143 references to the literature.

From the Authors' Summary.

Primary Carcinoma of the Lung. K. B. Olson, Am. J. Path. **11**:449, 1935.

Sixty-nine cases of primary carcinoma of the lung, verified at autopsy, have been presented and divided into three groups: (*a*) squamous cell carcinoma, (*b*) adenocarcinoma and (*c*) undifferentiated carcinoma.

Squamous cell carcinoma constituted the largest single group and 42 per cent of the entire series. The left lung and upper lobes were the most common site of the primary tumor, and in 61 per cent of the cases it involved a bronchus. Cavitation in the primary tumor occurred in 17 per cent. Metastases and extensions were not so widespread as in the undifferentiated group but were more extensive than in that of adenocarcinoma.

Adenocarcinoma constituted 24 per cent of the series and was mucinous in 53 per cent of the cases and nonmucinous in 47 per cent. In all cases the tumor probably originated from the epithelium lining a bronchus or from a peribronchial mucous gland. The mucinous type frequently metastasized and occasionally extended but appeared less malignant than the nonmucinous. It involved bone more frequently than any other type. The nonmucinous type was the least malignant and was occasionally confined to a lobe or a lung. It frequently involved the pleura.

Undifferentiated carcinoma constituted 33 per cent of this series. The primary tumor occurred slightly more frequently in the left lung, always involved a bronchus and occasionally infiltrated an entire lung. It showed the most vigorous tendency to metastasize widely and to extend locally. All the cases occurred in the left lung, in the upper lobes and at the hilus. The primary tumor was a single mass in 95.7 per cent of the cases and usually involved or occluded a bronchus. This type of carcinoma metastasized widely, and the primary tumor was very prone to extend regionally. Skeletal and intracranial metastases were common.

An absolute increase in the general incidence of carcinoma of the lung occurred at the Boston City Hospital in the period from 1930 to Aug. 1, 1934, and is possibly explainable as a selective phenomenon. Males were affected predominantly, in the ratio of 4.5:1. The incidence in males has increased in the past fifteen years. The majority of cases occurred in the sixth and seventh decades of life. Adenocarcinoma tended to occur more frequently at the extremes of life. Asso-

ciated pulmonary inflammatory conditions occurred in 58.8 per cent of the cases. The incidence of pulmonary tuberculosis and pneumonoconiosis in this series was consistent with the incidence in unselected cases.

ADENO-ACANTHOMA OF THE PYLORUS. J. G. PASTERNACK, Am. J. Path. **11**: 541, 1935.

A case of cornifying epidermoid carcinoma occurring with an adenocarcinoma of the pylorus in which definite transitions from glandular to epidermoid carcinoma were present is reported. The tumor removed at operation was predominantly epidermoid and was confined to the pylorus. No metastases or other tumor foci were demonstrable. At autopsy, the esophagus and cardia were normal, the tumor in the vicinity of the gastric resection was predominantly adenocarcinomatous, and the omentum, lymph nodes and pancreas were infiltrated only by adenocarcinoma.

FROM THE AUTHOR'S SUMMARY.

ERYTHROBLASTOSIS. G. W. COVEY, Am. J. Path. **11**:551, 1935.

A brief review of the pertinent literature is given with special reference to the relation between erythroblastosis, erythroleukoblastosis and fetal leukemia. Attention is drawn to the fetal mechanism apparently designed to meet oxygen-poor conditions of intra-uterine life, an erythremia with a large number of immature red blood cells, and to their rapid reduction in number immediately following birth. A possible analogy is pointed out between erythroblastosis and fetal leukemia, on one hand, and adult polycythemia vera and leukemia on the other. The probable rôle of fetal hydrops and icterus gravis neonatorum as complications of erythroblastosis in a new-born infant having an erythroblastoma in the left pleural cavity is reported, with the observations at autopsy and a description of the histopathologic changes.

FROM THE AUTHOR'S SUMMARY.

THE EFFECTS OF GAMMA RAYS OF RADIUM AND OF ROENTGEN RAYS ON LYMPHOMATOSIS OF MICE. J. FURTH and D. H. KABAKJIAN, Am. J. Roentgenol. **32**:227 and 377, 1934.

Continuous exposure of mice with transmitted lymphomatosis to the gamma rays of radium does not prevent the fatal termination of the disease but often prolongs life. Malignant lymphocytes introduced into healthy mice continue to grow under constant exposure to quantities of radiation that cause fatal damage to the host. The gamma rays of radium increase the susceptibility of mice to transmitted lymphomatosis.

Total irradiation of lymphomatous mice by roentgen rays prolonged their life. This effect was proportional to the quantity of roentgen rays applied. Fractional irradiation also prolonged the life of leukemic mice, but under the conditions prevailing in these experiments it was not more effective than single irradiation with a correspondingly large dose. The prolongation of life of leukemic mice by roentgen rays is due to a direct effect of the roentgen rays on the malignant lymphocytes. The death rate from intercurrent diseases was greater among the irradiated than among the nonirradiated animals.

FROM AUTHORS' CONCLUSIONS.

BIOPSY IN BONE SARCOMA. JAMES EWING, Am. J. Surg. **27**:26, 1935.

After discussing the objections to typical biopsy in bone sarcoma and its shortcomings, Ewing states that biopsy of material obtained by aspiration with an 18 bore needle has been remarkably successful in revealing the structure of bone tumors. By passing the needle through a minute incision in the skin, after the application of a drop of procaine hydrochloride, it is usually easy to penetrate the shell of a medullary tumor and secure by suction enough tissue to yield a

positive diagnosis. With a soft extramedullary tumor the method is practically always satisfactory. When the operator acquires some skill, and the pathologist is willing to employ care and patience, a very high proportion of correct diagnoses may be obtained. Most of the objections to the surgical biopsy are avoided. When this method becomes a familiar routine there remains only a very restricted field for the surgical biopsy.

STRUCTURE AND BEHAVIOUR OF THE CELLS IN TISSUE CULTURES OF TUMOURS. R. J. LUDFORD, Scient. Rep. Invest. Imp. Cancer Research Fund **11**:147, 1934.

Malignant cells of different strains vary in size, in structure, in degree of differentiation and in their manner of growth in mediums which support the growth of normal cells. They are less actively motile than polyblasts. Their cytoplasm is more finely granular than that of normal cells, their nuclei relatively larger, and the mitochondria usually smaller. Abnormalities of mitosis are common. They are not stained vitally with neutral red so intensely as polyblasts. Most malignant cells fail to stain vitally with trypan blue, like their normal prototypes. The rate of growth of malignant cells of the various strains of transplantable tumors in vitro is correlated with their rate of growth in vivo. Malignant cells retain the same cytologic features in vitro as in vivo. Significant differences occur in the growths of different strains of mouse tumors in mouse and rat serum. Explants of some strains grow as large sheets of malignant cells in both serums, others will not in either medium, while some give excellent sheet growths in mouse serum but not in rat serum.

THE REACTION OF NORMAL AND MALIGNANT CELLS TO FAT-SOLUBLE COLOURED COMPOUNDS WHICH ARE INSOLUBLE IN WATER. R. J. LUDFORD, Scient. Rep. Invest. Imp. Cancer Research Fund **11**:169, 1934.

Relatively stable colloidal solutions of the fat-soluble, water-soluble colored compounds sudan III and sudan black have been made in serum. Serum colored in this manner, sometimes with trypan blue added, has been applied to tissue cultures of normal and malignant cells. Fat droplets in both normal and malignant living cells are colored specifically—a vital histochemical reaction. Fat droplets in dead and fixed cells stain in the same manner with the colored serum. The application of a mixture of sudan III and trypan blue in serum to tumor cultures (Crocker sarcoma, mouse carcinoma 206) results in the fat droplets being stained in both the normal and the malignant cells, but in these experiments only the polyblasts have segregated trypan blue. Fat droplets in fibroblasts are colored by sudan III, and small, faintly colored droplets are present in cultures of embryonic fibroblasts to which sudan black has been added. Vitally stained fat droplets occur in dividing cells. Flocculated and precipitated particles of sudan III and sudan black are segregated, and also phagocytosed, by polyblasts. These results suggest that, although malignant cells are readily permeable to fat-soluble substances, they are less permeable to water-soluble compounds than are normal cells. The tentative explanation is put forward that the plasma membrane of malignant cells is relatively rich in fatty substances.

FROM THE AUTHOR'S SUMMARY.

BILE PIGMENT FORMATION IN THE METASTASES OF A CARCINOMA OF THE LIVER. N. TOKOZAWA, Centralbl. f. allg. Path. u. path. Anat. **61**:3, 1934.

Evidence is adduced from the following case to indicate the site of the transformation of hemoglobin into bile. A laborer, 45 years old, a moderate consumer of alcoholic drinks, noticed distention of and pressure in the upper part of his abdomen and within a month became bed-ridden. Repeated paracenteses yielded bloody ascitic fluid. Intermittently a subicteric tinge was noted in the ocular conjunctiva, but the skin remained free from icterus, and the bilirubin reaction of the urine was constantly negative. Death occurred about six months after the onset

of symptoms, and at necropsy there were found: emaciation; a hemorrhagic ascites of 10,000 cc.; a primary carcinoma of the quadrate lobe of the liver, the size of a hen's egg; many dark green cancer metastases of the great omentum and visceral peritoneum, and cirrhosis of the liver. The evolution of the primary growth could be traced through cirrhosis to nodular hyperplasia and adenoma formation to carcinoma. Bilirubin was found in abundance in the reticulo-endothelial cells of the metastases. In contrast to this it could be demonstrated only feebly in the tumor cells. The possibility that the bilirubin in the reticulo-endothelial cells occurred as a matter of storage is discredited because there was no dilation of bile capillaries in the tumor or in the cirrhotic liver and no bile cylinders in the gall capillaries to indicate bile stasis. There was, also, no clinical evidence of icterus except occasionally in the bulbar conjunctiva. The blood serum at no time contained quantities of bilirubin indicative of icterus, and a similar negative condition was found in the urine. The author feels justified in concluding that the bilirubin in the reticulo-endothelial cells of the metastases is made in loco and that this case is a contribution to the anhepatocellular theory (Aschoff) of the formation of bilirubin.

GEORGE RUKSTINAT.

ASSIMILATORY GROWTH OF CARCINOMA. W. SCHILLER, Virchows Arch. f. path. Anat. **292**:577, 1934.

The normal course and arrangement of the epithelial fibrils from the normal to the carcinomatous epithelium observed at the advancing margin of squamous cell carcinomas of the vulva and cervix uteri are interpreted by Schiller as evidence of the transformation of normal epithelium into carcinomatous cells. For such an increase in the size of a carcinoma he prefers the term "assimilatory growth" to "appositional growth."

O. T. SCHULTZ.

TUMORS OF THE RETE OVARII. J. WALLART and S. SCHEIDEGGER, Virchows Arch. f. path. Anat. **292**:643, 1934.

The authors believe that the rete ovarii is derived from the portion of the wolffian duct that gives rise to the primitive kidney. They believe also that the structure is not a useless embryonic rest, but a structure that functions actively in the sexual activities of woman, although the nature of its function is unknown. They describe an ovary removed from a 46 year old woman. The rete had undergone adenomatous proliferation, all transitions from normal to adenomatous structures being evident. In some of the branching tubular structures the epithelium was multilayered, but in general it consisted of a single layer of very tall columnar cells. The epithelium formed papillary ingrowths into the lumen of some of the spaces. In a second case, one of primary bilateral carcinoma of the ovary with metastasis in a woman aged 61, the origin is also ascribed to the rete, on the basis of the character of the epithelium and of the papillary structures formed by it.

O. T. SCHULTZ.

Technical

CONFIRMATORY TEST FOR SYPHILIS OF WITEBSKY. G. D'ALESSANDRO and F. SOFIA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **83**:478, 1934.

Witebsky's confirmatory test (abstr., ARCH. PATH. **18**:749, 1934) is based on a liberation of the antibodies from the precipitate which develops in a flocculation test for syphilis. The solution containing the liberated antibodies, which is almost entirely free from protein, is then used for a complement-fixation test. The use of a 1:1,000 dilution of solution of formaldehyde in a physiologic solution of sodium chloride eliminated certain technical difficulties of the original procedure. The test eliminated some of the false positive reactions. In a few cases of mixed syphilitic and gonorrheal infection and of mixed syphilitic and tuberculous infection

the serum from which the syphilitic antibodies were removed fixed complement specifically with the homologous gonorrheal or tuberculous antigen. False positive reactions with serums of patients with malaria could not be eliminated by means of the test. However, a differentiation was possible when an alcoholic extract of human red blood cells was used as the antigen. The antibody-containing solution of the confirmatory test reacted more intensely with the red cell antigen in cases of malaria and vice versa in cases of syphilis, while the untreated serum failed to show any differences with the two antigens. The test was positive in those cases of treated syphilis in which the Wassermann reaction with the untreated serum was positive and the flocculation test negative. Addition of normal serum which had been treated with normal hydrochloric acid changed the solution containing syphilitic antibodies in such a manner that it reacted positively in the precipitation test but negatively in the complement-fixation test.

I. DAVIDSOHN.

THE CITOCHOL FLOCCULATION TEST FOR SYPHILIS. R. SCHMIEMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:64, 1934.

The new modification of the Sachs-Witebsky citochol test which employs 0.1 cc. of serum and 0.1 cc. of a 1:12 dilution of a 3 per cent solution of sodium chloride proved more sensitive than the old technic which used 0.025 cc. of extract in a dilution of 1:3 with physiologic solution of sodium chloride. Schmiemann found that the new technic permits the use of one-half the amount of serum and that it could be successfully adapted for a microscopic slide technic, though there is hardly a need for it. By the use of proper dilutions of serum, the citochol reaction can be well employed as a quantitative test.

I. DAVIDSOHN.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

May 13, 1935

I. Pilot, M.D., *Presiding*

Edwin F. Hirsch, M.D., *Secretary*

A Clinical and Experimental Study of the Macrophage System of the Lungs in Relation to Recovery from Lobar Pneumonia. O. H. Robertson and Lowell T. Coggeshall.

In an earlier study of the pathology of experimental pneumococcic lobar pneumonia in the dog certain striking histologic changes were observed constantly in the involved lung at the time of recovery, the nature of which suggested the occurrence of local or tissue immunity. In order to determine whether similar changes took place in the human lung in lobar pneumonia a postmortem study was made of the tissues of forty patients dying at intervals of from six to twelve hours to two months after the onset of the disease in whom the approximate age of the different lobar lesions had been determined by x-ray pictures made daily during life.

Histologic changes analogous to those seen in the dog's lung were found wherever resolution was taking place. Often the evolution of the whole process could be followed in a single case in which lesions of different ages were present. The first evidence of reaction consists of an increase in the number of large mononuclear cells in the alveolar walls, many of which protrude into the air spaces. This results in a thickening of the septums. As the process develops the large mononuclear cells become detached from the alveolar wall and enter the exudate where they exhibit the form and phagocytic functions of the macrophages. The latter cells gradually replace the polymorphonuclears, the fibrin disappears progressively and the lesion assumes the characteristic appearance of resolution. The same type of tissue cell reaction was observed in the lymph glands at the hilus of the lung.

Sections obtained from six patients who died at intervals of from six days to two months following recovery from lobar pneumonia showed a pronounced macrophage reaction.

The most striking observation in the study of these human tissues was that wherever a well developed macrophage reaction occurred pneumococci were few or absent while in most lesions of all ages in which the exudate was predominantly polymorphonuclear leukocytes micro-organisms were abundant. Such marked differences in numbers of pneumococci were observed not only between lobar lesions but also at times in different parts of the same lesion where focal macrophage reactions were occurring. The macrophages were seen to be actually phagocytic and showed evidence of effective digestion of the engulfed pneumococci.

Further data on the significance of this reaction were obtained experimentally from certain animals in which clearing of one part of the pneumonic lesion occurred while spreading took place in another part of the lung. The lungs from such animals put to death during the active phase of the disease yielded sterile cultures from the clearing lesion which showed a marked macrophage reaction, while the new lesions contained many pneumococci and exhibited the histologic appearance of a spreading process. Other experimental evidence was presented to indicate that the macrophage reaction represents an immune response.

DISCUSSION

P. R. CANNON: What is the nature of the mononuclear phagocytes?

S. C. PEACOCK: Are patients with chronic bronchitis or other chronic infection of the lungs less susceptible to lobar pneumonia?

O. SAPHIR: May the presence of macrophages in the gray stage of lobar pneumonia result from organization changes?

O. H. ROBERTSON: The phagocytes have been vitally stained, and most of the cells in the gray stage of pneumonia are macrophages. Patients with chronic bronchitis infrequently contract lobar pneumonia. This may have some relation to the presence of macrophages in these chronic infections. Much evidence indicates that macrophages are concerned with resolution rather than organization. In the latter, fibroblasts are present. The resolving process was studied thoroughly in dogs examined in various stages of lobar pneumonia.

EFFECT OF DISSOCIATION OF STREPTOCOCCI ON THEIR FIBRINOLYTIC AND ANTIFIBRINOGENIC ACTIVITY. RUTH TUNNICLIFF.

Tillett and Garner (*J. Exper. Med.* **58**:485, 1933) have made the interesting observation that broth cultures of the beta hemolytic streptococcus of human origin rapidly dissolve the normal human fibrin clot. With the exception of some strains of Staphylococcus aureus other bacteria, including Streptococcus viridans, the pneumococcus and most hemolytic streptococcus strains of animal origin, have not been demonstrated to have this property of fibrinolysis.

Hadfield, Magee and Perry (*Lancet* **1**:834, 1934) found greater variation in the dissolving power of their strains of the hemolytic streptococcus than has been described by others. They observed that strains virulent for mice, which formed colonies corresponding to those called smooth in this country, had the strongest fibrinolytic property. As their strains became less virulent they became less fibrinolytic.

For my tests, strains of the hemolytic streptococcus were grown twenty hours in meat extract 1 per cent dextrose broth, p_H 7. If they would not grow in this medium, 1 drop of defibrinated sheep blood was added to 5 cc. of dextrose broth.

The method of Tillett and Garner was followed. Since small amounts of blood were more convenient the blood was collected from the finger or ear into a capillary pipet containing 2 per cent sodium citrate in salt solution. Two tenths per cent potassium oxalate gave the same results. Two parts of blood were drawn into a pipet containing 1 part of the anticoagulant and the mixture diluted at once with 8 parts of the physiologic solution of sodium chloride and centrifugated. Four parts of the diluted plasma were then mixed with 2 parts of broth culture and 1 part of 0.25 per cent calcium chloride solution in small test tubes. The mixtures were incubated at 36-37 C. The control containing plasma, uninoculated broth and calcium chloride clotted in from five to fifteen minutes. When the broth cultures dissolved the plasma clot this occurred in from fifteen minutes to twenty-four hours after its coagulation.

Seventeen hemolytic streptococcus strains from scarlet fever, erysipelas, septic sore throat, malignant endocarditis, septicemia and sore throat were examined for their dissolving activity on normal human plasma clot. All dissolved the clot. I find that the strongest fibrinolytic activity is associated with the virulent strains which have capsules and produce smooth colonies. This property is gradually lost as the cocci form more granular colonies and finally colonies that are dull, rough and flat with irregular, lacy edges, and as they change from round diplococcic forms into the large round or flattened cocci in twisted chains or into the bacillary and filamentous forms characteristic of the rough type of streptococcus. They generally lose their dissolving power before they are completely stabilized as rough. As the rough forms revert to the smooth, morphologically and colonially, the cocci again acquire the power to dissolve the fibrin clot.

My observations are in accord with those of Dennis and Berberian (*J. Exper. Med.* **60**:581, 1934) that strains of Str. viridans do not dissolve the fibrin clot but may inhibit its formation. This property seems to be associated with the smooth type of colony. I examined five strains from subacute and malignant endocarditis, two from measles and one from sore throat. One strain of a non-hemolytic streptococcus from endocarditis behaved like the greening cocci. Like the hemolytic streptococcus, the green-producing streptococcus associates virulence with the smooth type of colony. As the cocci of smooth strains change into those of the rough type they lose virulence and show the same morphologic and colonial variations as are seen in rough cultures of the hemolytic streptococcus. When they revert to the smooth form of colony they also revert morphologically. I find that the anticlotting property of Str. viridans is associated with the smooth virulent type of colony and as the cocci change into the rough form they lose this property, but reacquire it as they revert to the smooth type.

Although the greening colonies may appear smooth for several days, if the broth culture does not prevent clotting of the plasma rough projections with their bacillary forms will be seen at the edge of the colony, generally within a week.

To determine whether a streptococcus broth culture is of the smooth or of the rough type it is advisable to plate onto blood agar and examine with a culture microscope for rough colonies.

Summary.—The fibrinolytic activity of the hemolytic streptococcus appears to be associated with the virulent smooth type of colony. The power is lost as the colony becomes rough, and is regained as it reverts to the smooth form.

Str. viridans does not have power to dissolve the fibrin clot but may prevent its forming. This antifibrinogenic activity is associated with the virulent smooth type of colony, is lost as the colony changes to the rough avirulent form, and is regained as it reverts to the smooth type.

DISCUSSION

O. H. ROBERTSON: Have you studied the fibrinolytic and antifibrinogenic properties of various immune serums?

R. TUNNICLIFF: I have made no such studies.

RENAL DENERVATION: EFFECT OF DAILY INJECTIONS OF COLON BACILLI AND PITRESSIN ON THE DENERVATED KIDNEY OF THE DOG. GEORGE MILLES and MAURICE HARDGROVE.

The article appeared in full in the October 1935 issue of the ARCHIVES, page 548.

DISCUSSION

H. JAFFE: These experiments should be tried on animals that are not so prone to spontaneous fibrosis of the kidneys.

G. MILLES: We consider the experiments adequately controlled even though dogs only were used.

CYANOTIC ATROPHY OF THE LIVER: A WAX MODEL RECONSTRUCTION. C. S. HAGERTY and J. W. DEVEREUX.

This article will be published in full in the ARCHIVES OF PATHOLOGY.

SUPPURATIVE AORTITIS: REPORT OF TWO CASES. MARSHALL Q. BAKER.

Two cases of suppurative aortitis are described, both associated with pre-existing syphilitic aortitis.

Rupture of the supravalvular portion of the aorta with hemopericardium occurred in one. The causative micro-organisms (pneumococci and streptococci) were demonstrated and the probable pathogenesis was indicated in each one.

The condition has never been diagnosed clinically, always being discovered incidentally post mortem.

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, May 24, 1935

KORNEL TERPLAN, *President, in the Chair*

W. F. JACOBS, *Secretary*

CARCINOMATOUS CIRRHOSIS OF THE LIVER WITH SARCOMATOSIS OF THE PERITONEUM. S. SANES and E. F. COOK.

A case is reported because of the occurrence of two different types of malignant neoplasm with typical atrophic cirrhosis of the liver. That a pathogenic relationship exists between Laënnec's cirrhosis and primary carcinoma of the liver is generally recognized. Whether the association of an endothelial sarcoma of the peritoneum with the cirrhosis in this case was more than a coincidence seemed an interesting point for discussion.

An Italian, 57 years of age, was admitted to the hospital on Nov. 29, 1934. All his adult life he had partaken of wine and whisky daily. He first began to lose weight and strength in 1932. In March 1934 he complained of cramplike abdominal pain, diarrhea and bloating. The liver and spleen were distinctly palpable; the legs were edematous. Fluid was demonstrated in the abdominal cavity. Urobilinogen was present in the urine; the van den Bergh reaction was positive. In the last six months of the patient's life fluid accumulated rapidly in the abdominal cavity. Samples obtained by paracentesis showed no tumor cells. The patient died rather suddenly.

The anatomic diagnosis was: marked Laënnec's cirrhosis of the liver with typical small-nodular regeneration; chronic splenic tumor (600 Gm.); varices of the lower esophageal veins with recent rupture in one vein; marked gastroenterorrhagia; slight icterus of the skin; multiple carcinomatous nodules in the liver, ranging from walnut size to small peach size and showing distinct necrosis and icterus (cirrhosis carcinomatosa); primary endothelial sarcoma of the omentum, mesentery and entire visceral and parietal peritoneum; hemorrhagic ascites (2,000 cc.).

The omentum, mesentery, visceral and parietal peritoneum were studded with tumor nodules ranging from pea size to cherry size. Many were pedunculated and hemorrhagic. Their surfaces were smooth; their consistency, firm; their color, grayish. Several nodules floated in the ascitic fluid. Where the peritoneum presented no nodules it was opaque. No metastases were found in lymph nodes. Histologically the tumor proved to be an endothelial sarcoma. In uninvolved areas of the peritoneum there was a distinct proliferation of serosal cells. They were increased in size and formed several layers. The subserous tissue was distinctly thickened. It contained many dilated lymphatic vessels. A few of these were lined with hyperplastic endothelial cells. The tumor nodules themselves disclosed various stages of growth. In the immature type the cells varied in shape and size. They were predominantly fusiform or polyhedral. Syncytial processes could be seen. Uninucleated and multinucleated giant cells were present in abundance. Silver impregnation stains revealed reticulum fibers in the stroma. The cells in the mature type were practically only fusiform; they appeared to be arranged in bundles. Small capillary vessels were prominent. Invasion of the outer muscle layer of the intestine had taken place.

DISCUSSION

K. TERPLAN: Macroscopically this case was first a diagnostic problem. The primary character of the multiple nodular tumors of the mesentery and omentum was readily recognized. The soft tumor nodules in the cirrhotic liver, however, were first thought to be metastases of the peritoneal sarcoma. Although the

combination of these two different malignant blastomas in a case of Laënnec's cirrhosis is certainly most unusual and known to few, if known at all, the temptation is great to look for some pathogenic relationship between this primary sarcoma of the peritoneum and chronic ascites from atrophic cirrhosis. In chronic ascites from portal obstruction distinct thickening of the visceral and parietal peritoneum, so-called plastic peritonitis, is usually observed. It is believed that this proliferation of the mesothelial and subserous mesenchymal cells is a resorptive (?) effect of chemical (toxic) irritation rather than of continuous pressure from the large volume of fluid alone. Thus this prolonged irritation of the lining surface of the peritoneal cavity could be considered as *one* factor bringing about, besides mere hyperplastic thickening of the serosal cells, true blastomatous proliferation. A second point of interest is that lymphogenic and hematogenous metastases from the primary peritoneal sarcoma were not discovered.

PATHOLOGY OF YAWS, ESPECIALLY THE RELATION OF YAWS TO SYPHILIS. H. U. WILLIAMS.

This article appeared in full in the October 1935 issue of the ARCHIVES, page 596.

FAT CONTENT IN THE BLOOD OF THE RIGHT SIDE OF THE HEART IN A CASE OF FATAL FAT EMBOLISM. K. TERPLAN, R. S. HUBBARD and C. T. JAVERT.

This case is presented for two reasons: The diagnosis of fat embolism was made on gross inspection of the blood in the right side of the heart, and the fat content of the blood from the right ventricle was quantitatively determined.

A white man, 70 years of age, was injured in an automobile accident. A fever of 101 F. developed; the pulse rate was 80; the respirations 18; the blood pressure 62 systolic and 40 diastolic. The fever rose to 105 F., and the patient died about four hours after the accident. The chief observations post mortem were: a depressed fracture of the frontal bone, fractures of the seventh cervical and the seventh to tenth thoracic vertebrae, multiple fractures of the right femur and patella, minor contusions and lacerations, subarachnoid hemorrhage and lacerations of the frontal lobe. The right side of the heart and the pulmonary artery contained fluid blood with innumerable fat globules on its surface. The fluid blood in the left ventricle and aorta did not contain visible fat. The foramen ovale was closed. The lungs felt greasy to palpation. Fat was readily demonstrated in the pulmonary capillaries in stained sections but not in the brain or in the blood obtained from the left ventricle.

Blood taken from the right ventricle and pulmonary artery was chemically analyzed. The specimen, measured in a graduated cylinder, consisted of approximately 8 cc. of a mixture of gross fat and partially hemolyzed blood. There was no evidence of the presence of an emulsion, as drops of fat were floating on the surface of the liquid.

The material and container were repeatedly extracted with a mixture containing 3 parts of alcohol and 1 part of ether. The extract was evaporated to dryness and the residue taken up with ether. After further purification by repeated evaporation and resolution in ether the extracted lipoids were dried on a water bath and brought to constant weight in a desiccator. The lipoid recovered weighed 1.251 Gm.

One and one-tenth grams of the material was found to be in the form of "total fatty acid" (Bloor, W. F.; Pelkan, E. F., and Allen, D. M.: *J. Biol. Chem.* **52**:191, 1922). Titration with sodium alcoholate in benzene showed that none of this was in the free form. Also present were 0.67 mg. of organic phosphorus (Harnes, A. R.: *J. Biol. Chem.* **77**:405, 1928) and 11 mg. of cholesterol (Bloor, W. R.: *J. Biol. Chem.* **24**:227, 1916). The iodine number (Ralls, J. O.: *J. Am. Chem. Soc.* 121, 1934), corrected for the cholesterol, was 55.3. This corresponds to that of a mixture of glycerides of stearic and oleic acid containing 64 per cent triolein.

There was less pigment soluble in petroleum ether than was found in a comparable amount of fat extracted from normal blood. There was, however, a small amount of a pigment present which was not found in fat from normal blood. This pigment was yellow-brown and was easily soluble in ethyl ether but insoluble in petroleum ether.

Blood from the right ventricle of a patient dying from fat embolism contained between 15 and 16 Gm. of fat per hundred cubic centimeters of fluid. The extra lipoid was in the form of a neutral fat with a fairly low iodine number.

Obituaries

EDWIN RAYMOND LeCOUNT

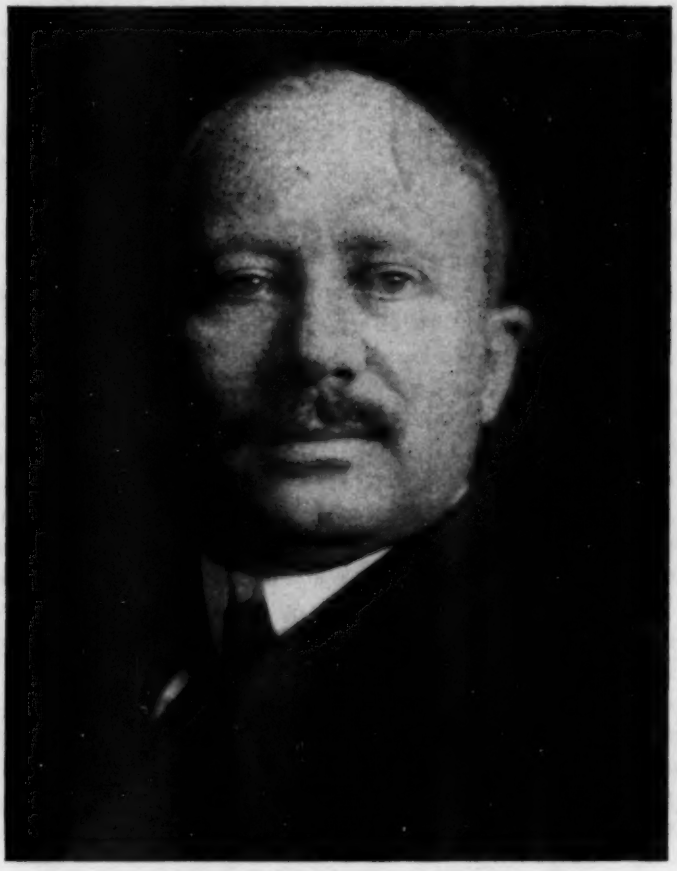
1868-1935

Edwin Raymond LeCount was born in Wisconsin on April 1, 1868. He entered Rush Medical College from Carroll College at Waukesha, Wis., in 1889 and received his medical degree in 1892. A year and a half later he completed his internship in the Cook County Hospital.

At that time the instruction in pathology in Chicago was at the best rudimentary and superficial. In the hospitals there were no pathologic examinations with one glorious exception: the demonstrations by Christian Fenger in the necropsy room of the Cook County Hospital and in connection with his surgical clinics. By arousing interest in pathology and in a deeper understanding of disease, this master pathologist profoundly influenced the ideals and careers of many young physicians, including Dr. LeCount. There was then hardly any sustained or systematic medical research of any kind in Chicago; with rare exceptions, the output consisted of reports of clinical observations. But the end of a period in American medicine was at hand; a new era was coming with new facilities and fresh enthusiasm. Putting his trust in the future, Dr. LeCount, soon after his internship, began working in pathology at Rush Medical College, which he was to serve faithfully to the end of his days. Since about 1902 he had charge of the work in pathologic anatomy.

He at once set to work to strengthen his fundamental training. He developed a concise and orderly style of writing. He mastered French and German when he realized his need of direct access to the medical literature in these languages. He spent several months in Dr. Welch's laboratory in the Johns Hopkins Hospital. He acquired and maintained a remarkably good understanding of the chemical, physical and microbiologic factors in pathologic morphology. His two periods of study in Europe were centered, one on microbiology at the Pasteur Institute in Paris in 1896, and the other on chemistry in Berlin and Halle in 1905. He became essentially a pathologic anatomist of the classic type, devoted primarily to teaching and advancing the knowledge of the morphologic aspects and genesis of the structural changes in disease and injury. He accumulated a rich and varied experience in the laboratory as hospital pathologist and as physician to the coroner's office of Cook County for thirteen years (1911 to 1924). He set a high standard for necropsy and for necropsy records. In connection with his medicolegal work, he developed a well organized system of volunteer assistantships. The duties

were indeed arduous, but the opportunities to learn were so attractive that there never was any lack of acceptable applicants. The work began between 4 and 5 o'clock in the morning, so that it might be finished before the hour of the regular course in pathologic anatomy. So tireless was Dr. LeCount's industry, so fully had he taken himself in hand, that during these years he rose shortly before 4 o'clock. In the course of



EDWIN RAYMOND LeCOUNT
1868-1935

his medicolegal service he gathered a remarkable series of records of cases (99 volumes, fully indexed), which form a copious source of information concerning the pathologic anatomy of medicolegal conditions. He was engaged in the study of this material to the last, and in his room in the hospital he completed valuable analyses of cases of fractures of the skull and of gunshot wounds involving the chest and abdomen simultaneously.

As the years passed he developed into an authority in practical pathology, clinical and medicolegal. His experience, the skill and thoroughness of his examinations, his wide knowledge, his refusal to come to any final decision in the absence of conclusive evidence and the soundness of his judgments gave him the standing of a supreme judge in questions of diagnosis on a structural basis.

The plan Dr. LeCount eventually followed in teaching pathologic anatomy was in brief to give each student full opportunity to learn through his own efforts—no so-called spoon feeding. "The materials are before you. What do you see and what does it mean? Here are references to the literature." It was an earnest effort to get the real work of the course done by the student himself. He frequently told his students that he would not "rob them of the joy of discovery." To many students the plan was not immediately acceptable; they expected to receive more direct instruction, to be told more about what they were supposed to discover and to interpret. Many were not satisfied with their own efforts. Perhaps the method was too difficult, at least in some cases. But on the whole a consensus developed, which grew stronger with time, that the method was far more effective than anticipated. "He made me think," "he taught me to see for myself," "he trained me to discover morbid changes independently" are comments that illustrate the feeling of former students as time lengthened their perspective. No higher praise could be given, and the records of his students and assistants leave no doubt of the great value of his teaching. He was tireless in his efforts to arouse in them the spirit of investigation. Indeed, his whole plan of instruction was based on independent effort. Probably not a single class completed his course without one or more members being singled out for special work, usually on remarkable cases or on some concrete problem. The "Transactions of the Chicago Pathological Society" contain many reports of such work. Many clinicians and pathologists, including not a few of both groups who are now in important academic positions, received through him the introductory investigative stimulus. The value of this influence in the promotion of research cannot be overestimated.

Significant of Dr. LeCount's deep concern in research at an early stage in the medical career is his gift in 1931 of $10,000 to the Institute of Medicine of Chicago "for a trust fund to bear the name of Joseph Almarin Capps, with the provision that the income from the fund be used to establish a prize to be awarded each year for the most meritorious investigation in medicine in Chicago completed within two years after graduation by a graduate of a medical school in Chicago." So far four Capps prizes of $500 each have been awarded.

Dr. LeCount himself made important contributions to pathology. His studies of the microscopic lesions in Rocky Mountain spotted fever, based

on material furnished him by Howard T. Ricketts, and of the earliest changes in the lungs in influenza are especially noteworthy. He recorded observations of great value on coma, injuries and embolism. In 1934 the medical faculty of the University of Cincinnati bestowed on him the James E. Stacy Award for "his experimental studies on the isolation of streptococci from sore throats and the experimental induction, through their injection, of acute, healing and scarring types of nephritis, identical with forms of chronic nephritis observed in man."

Dr. LeCount dealt earnestly with facts and situations, and he pursued his objectives in a distinctive and determined way. He was also of a highly sensitive nature and subject to reactions of a defensive tendency, which sometimes may have been misunderstood. In the midst of the day's work he might be blunt and abrupt, but beneath the surface beat a warm and kindly heart. Children loved him instinctively, and he liked old people. He was unassuming, generous, helpful. To his friends he was frank and finely loyal. Sincerity, high standards and an underlying idealism guided his course.

LUDVIG HEKTOEN.

Book Reviews

Unfall und Hirngeschwulst: Ein Beitrag zur Aetiologie der Hirngeschwülste. By Prof. Dr. Otto Marburg, Vorstand des Neurologischen Institutes der Wiener Universität. Price, 8.80 marks. Pp. 106, with 12 illustrations. Vienna: Julius Springer, 1934.

The question of the relationship of trauma to the subsequent development of neoplasms is one of great interest, particularly to the pathologist and those concerned with the legal aspects of medicine. In this short monograph Marburg is concerned with the relationship between injuries of the head and the development of tumors of the brain. Following a discussion of the neuroglia and its development he presents three cases of his own, one of a medulloblastoma of the cerebellum, one of a "polymorph celled" glioma and a third in which the exact nature of the tumor is not clear. In all three cases the manifestations of the tumor were preceded by an injury to the head.

The author then presents a review of similar cases reported in the literature. In all, he has collected about one hundred and forty-one instances in which some injury preceded the appearance of the cerebral neoplasm. Each case is briefly abstracted. Following a discussion of these cases Marburg considers at some length the pathogenesis of tumors of the brain, particularly gliomas, and the relationship of their development to trauma. His conclusion is that there can be no doubt of the connection between injuries of the head and the development of intracranial neoplasms. The influence of such trauma he believes may be exerted in two ways: Either the trauma may disturb an existing embryonic rest, causing it to undergo neoplastic changes, or the trauma may produce some structural change, a scar or the introduction of a foreign body, which may stimulate the tissues to neoplastic formation.

The presentation of this material, although a valuable expression of opinion from the leading Austrian neuropathologist, should not be accepted without serious consideration. The mere temporal relationship between a given injury and the development of a tumor does not establish the trauma as the causative factor. Such "post hoc" argument is among the most common and dangerous in medicine. Until definite proof, such as that persons who have suffered from severe injuries of the head are more subject to the development of intracranial tumors than other persons, is forthcoming, the thesis that such tumors may develop as a result of trauma must be considered as unproved. At the moment evidence seems to be pointing away from any real causative relationship between trauma and the development of neoplasms.

Die Hormonforschung und ihre Methoden. By Max Reiss, Dr. med., Dr. rer. nat., Privatdozent für pathologische Physiologie an der Deutschen Universität in Prag. Price, 19 marks. Pp. 415, with 26 illustrations and 7 charts. Berlin: Urban & Schwarzenberg, 1934.

Reiss, who was associated with Arthur Biedl for a number of years, set out to write a brief and compact book on internal secretion that would include (*a*) the established results and (*b*) the methods of physiologic investigation. The book was meant for the research worker. Another aim of the book was to include the methodological experience that accumulated in the course of years in Biedl's institute.

The results of physiologic, pharmacologic and chemical phases of hormonal research are discussed in the first part of the book in 261 pages. The thyroid, the parathyroids, the thymus, the hypophysis, the adrenals, insulin and the ovarian and testicular hormones are treated with a clarity and completeness that are admirably combined with economy of words and space. The book can be highly commended

for the sobriety of its approach and for its critical analysis of the difficult and, in places, vague subject. Still more valuable than the first and theoretical part is the second part of the book, which in 142 pages treats of the methods used in endocrinologic research. A number of methods are presented for each procedure, with a critical discussion of their merits and shortcomings.

Some readers will welcome the inclusion of earlier methods, not so much for their practical value as for reasons of historical interest. To have so many technical details compiled in a handy volume will prove helpful to many. The style of the author is very clear. Exhaustive bibliographic references and eighteen columns of a subject index are appended. A stimulating two-page discussion of the relation between endocrinologic research and medical practice concludes the book.

Contribution à l'étude de la variabilité du virus tuberculeux. By P. Denys. Pp. 90, with 6 illustrations. Louvain: Imprimerie Saint-Alphonse, 1935.

The general conclusions from this thesis are: The variability of a microbic species manifests itself principally in its morphologic, cultural and serologic characteristics, as well as in its pathogenic power. These are the different aspects of the problem of variability which have been studied with reference to the bacillus of tuberculosis. The experiments show that its attributes are highly variable, so that it is possible to transform a typical Koch bacillus to a bacillus which has all the characteristics of a paratuberculous saprophyte. In a study of the variations of B C G it has been found that it is possible to modify its cultural and biochemical characteristics and at the same time to induce it to acquire pathogenic power, no doubt rather low but superior to that of the nonmodified strain. It has not been possible to secure any evidence of a tuberculous ultravirus. This problem requires new investigations in which consideration should be given to recent work on spontaneous infections of guinea-pigs.

Books Received

LABORATORY MANUAL OF THE DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY, PEIPING UNION MEDICAL COLLEGE. Prepared under the direction of C. E. Lim, Head of Department. Second edition. Price, $1.50. Pp. 190. Peiping, China: Kwang Yuan Press, 1935.

EAR EXOSTOSES. Smithsonian Miscellaneous Collections, Vol. 93, No. 6, Publ. 3296. Aleš Hrdlička, Curator, Division of Physical Anthropology, United States National Museum. Price, 50 cents. Pp. 100, with 5 plates. Washington, D. C.: Smithsonian Institution, 1935.

REPORTS OF THE COMMITTEE UPON THE PHYSIOLOGY OF VISION, XIV: CHARACTERISTICS OF DICHROMATIC VISION WITH AN APPENDIX ON ANOMALOUS TRICHROMATIC VISION. Medical Research Council, Special Report Series, No. 200. F. H. G. Pitt. Price, 1s. 3d. Pp. 58. London: His Majesty's Stationery Office, 1935.

REPERTORIO SISTEMATICO DEI MICETI DELL-UOMO E DEGLI ANIMALI. Arturo Nannizzi. Price, 100 lire. Pp. 556. Siena: s. a. poligr. Meini, 1934.

RÖNTGENBEFUND UND PATHOLOGISCH-ANATOMISCHER BEFUND BEI LUNGENKRANKHEITEN. VERSUCH EINER KRITISCHEN VERGLEICHUNG. Dr. med. Max Versé, o. ö. Professor der allgemeinen Pathologie und pathologischen Anatomie, Direktor des pathologischen Instituts der Universität Marburg. In 2 volumes. Price, 18 marks. Part 1, text, pp. 96; part 2, atlas, with 144 illustrations. Berlin: Otto Elsner Verlagsgesellschaft, 1935.

VORKOMMEN UND VERBREITUNG DER THYREOTOXICOSE IN SCHWEDEN. ZUR GEOGRAPHISCHEN PATHOLOGIE DES MORBUS BASEDOWI UND VERWANDTER KRANKHEITEN. Thor Sällström. Pp. 296, with 52 figures. Stockholm: Klara Civiltryckeri A.-B., 1935.

THE WISTAR INSTITUTE STYLE BRIEF. A guide for authors in preparing manuscripts for the most effective and economical method of publishing biological research. Prepared by the cooperative efforts of the editors of journals published by the Wistar Institute and the staff of the Wistar Institute Press. Price, $2. Pp. 169, with 23 figures and 37 plates. Philadelphia: The Wistar Institute Press, 1934.